

Recent Research in the Field of 5-Hydroxytryptamine and Related Indolealkylamines

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List of Abbreviations

ACTH	Adrenocorticotrophic Hormone
ATP	Adenosine-triphosphate
BAB	1-Benzyl-2-methyl-5-methoxy-N, N-dimethyl-tryptamine (benzylanalogue of bufotenine)
BAS	1-Benzyl-2-methyl-5-methoxytryptamine (benzylanalogue of serotonin)
BOL	2-Bromo-lysergic acid diethylamide
CNS	Central Nervous System
DCA	Desoxycorticosterone acetate
DOPA	3,4-Dihydroxy-phenylalanine
DPN	Oxidized diphosphopyridine nucleotide
EC	Enterochromaffin Cell(s)
EDTA	(Ethylenedinitrilo) tetraacetic acid tetrasodium salt
EEG	Electroencephalogram
GABA	γ -Amino-butyric acid
GFR	Glomerular Filtration Rate
5-HIAA	5-Hydroxyindole acetic acid
5-HIAAl	5-Hydroxyindoleacetaldehyde
5-HT	5-Hydroxytryptamine
5-HTP	DL-5-Hydroxytryptophan
4-HIAA	4-Hydroxyindole acetic acid
4-HT	4-Hydroxytryptamine
4-HTP	DL-4-Hydroxytryptophan
IAA	Indoleacetic acid
LSD	D-Lysergic acid diethylamide
LSM	D-Lysergic acid morpholide
MAO	Monoaminoxidase
MBL	1-Methyl-2-bromo-lysergic acid diethylamide
MLD	1-Methyl-lysergic acid diethylamide
MSH	Melanocyte-stimulating Hormone
PIH	β -Phenylisopropylhydrazine
PAS	Paraaminosalicylic acid
RPF	Renal Plasma Flow
STH	Somatotropic hormone
T	Tryptamine
TP	Tryptophan
TPNH	Reduced triphosphopyridine nucleotide
UML	1-Methyl-D-lysergic acid butanolamide

1. Introduction

This review article is intended to give an account of the progress of research in the field of 5-hydroxytryptamine (5-HT) and related indolealkylamines in the last 6 to 7 years. Approximately 2000 papers have appeared on the subject during this period. We shall endeavour to review here the best from the more important ones. The reader is referred to previous review articles for papers preceding 1955.

Fervour in research on 5-hydroxytryptamine has been growing since 1947 and there are as yet no signs of fatigue. This makes the reviewer's task difficult in many ways and there is the risk of his work becoming out of date during the printing period. This report frequently makes critical comments on the interpretation of experimental data. Of course these comments reflect strictly personal opinions which have been consolidated during a period of nearly thirty years of investigation on the enterochromaffin cell system and 5-hydroxytryptamine. However as CROSSLAND [284b] rightly points out «the enthusiastic worker in any field tends to direct his critical faculty towards the work of others and away from his own, and readers may reasonably complain that the writer's own results and opinions are accorded a prominence out of proportion to their importance». Therefore those who wish «to preserve a sense of balance» are recommended to consult other reviews [730b, 734, 735, 890b, 246b, 284b] and some of the more important special papers.

2. The Enterochromaffin Cell System

All vertebrates and some groups of invertebrates possess a particular system of cells which contain specific granules characterized by several well-defined and distinctive chemical and physico-chemical features and exhibiting peculiar behaviour in histological fixation.

This system is known as the *enterochromaffin cell system* (EC system).

It now seems justifiable to attribute to the enterochromaffin system all cells which, after simple formol fixation, have granules capable of reducing ammoniacal silver nitrate solution (argentaffinity); of turning yellow or brown following treatment with bichromate or other oxidizing agents (chromaffinity); of coupling in alkaline medium with diazonium salts; of exhibiting yellow fluorescence in Wood's light and, finally, of combining, after mild oxidation, with thioindoxyls at alkaline pH levels to give reddish thioindigoid dyes [212, 1253, 912b].

Alcohol, acetone and all the usual histological fixatives lacking formol are unsuitable for preserving the distinctive histochemical characteristics of the specific granules.

At present the EC system, in its broadest extension, includes:

1) The typical EC of the gastro-intestinal mucosa, biliary ducts and pancreas of vertebrates. Typical EC are present in the alimentary tract of all classes

of vertebrates, with the single possible exception of *Cyclostomata*. According to recent research [386d], even *Teleostei*, or at least some species of these fishes, contain typical EC in their intestines.

2) The typical EC in the intestines of ascidians and of *Amphioxus lanceolatus*. In *Amphioxus* the EC are met only in the mid-gut and in the dorsal part of the hind-gut [474d].

3) The argentaffin cells of the mammalian prostate and urethra [958, 35, 477, 666].

4) The argentaffin cells of the lizard oviduct and the frog urinary bladder.

5) The argentaffin cells of the thymus of some birds [967] and reptiles [1256b].

6) The chromaffin cells of the posterior salivary glands of octopods and of the hypobranchial body of some prosobranchiate purple-yielding molluscs.

7) Some of the secretory cells of the cutaneous glands of amphibians, where these cells contain a chromaffin or argentaffin secretion [1251].

8) The chromaffin cells of the nematocysts, the stinging organelles of some coelenterates [542], and the argentaffin cells of the soft tissues of the coelenteron of another coelenterate, *Calliactis parasitica* [1256].

9) The argentaffin cells of the venous glands of scorpions, especially of *Leiurus quinquestriatus* [6].

10) Occasionally, the mast cells of rats and mice. As a rule the 5-HT in these cells cannot be made visible by the available histochemical methods but this may occur under particular circumstances, as in the case of mast cells in precancerous mouse skin [281, 982], or of mast cells of rats given injections of large doses of 5-HT for long periods [349].

To lengthen the above list it might be worthwhile examining the pineal gland of man and monkeys and the venous sac of wasps and hornets.

The EC of the gastro-intestinal tract and the glandular formations originating from it constitute the *EC system in the strict sense*.

It should be emphasized again that the only common characteristic of the cells grouped together in the EC system in its broadest extension is the positivity of some histochemical reactions.

If, as it seems to be, the positivity of colour and fluorescence reactions is only a matter of 5-HT concentration (minimum required concentration = approximatively 1 mg per g cells) [86], it follows that any substantial improvement in the sensitivity of histochemical methods will produce a conspicuous enlargement of the inhomogenous EC system, with the possible inclusion of not only normal mast cells of rats and mice, but also of blood platelets, nervous cells [761b] and other animal and vegetable cells as well.

It is now generally accepted that the cells of the enterochromaffin system owe their peculiar histochemical properties to their content of 5-HT and/or strictly related 5-hydroxyindole derivatives (as in amphibian skin) [364, 67, 912, 84, 367].

Evidence for this assumption is as follows:

a) All tissues containing cells belonging to the enterochromaffin system also contain remarkable amounts of 5-HT or kindred substances.

Generally, the content of 5-HT is strictly related to the number of enterochromaffin cells and the intensity of their histochemical reactions. When a tissue usually containing enterochromaffin cells is lacking in them, no 5-HT is found in the extracts of this tissue.

The above is true for the gastro-intestinal mucosa of vertebrates, the mast cells of mammals, the posterior salivary glands of octopods, the hypobranchial body of *Murex*, the cutaneous glands of amphibians, and the venous glands of scorpions.

b) The first appearance of EC in the intestinal mucosa of the embryonic calf coincides with the first appearance of 5-HT in extracts of its gastro-intestinal tract [394].

c) Reserpine, which is known to cause a release of 5-HT from the gastro-intestinal mucosa, also provokes a reduction in the number of EC, as well as a reduction in the richness of the specific granules and in the intensity of their specific histochemical reactions [221, 1253, 85, 86, 349, 957, 1254].

Following reserpine treatment, the decrease and return of 5-HT in tissue parallels the number of visible EC [85]. Similarly, the decrease in number, granular index and intensity of histochemical reactions of the EC in different species and different regions of the alimentary tract strictly parallels the decrease in the 5-HT content of intestinal tissue.

Reserpine has a rather poor action on the number and granular index of the EC of the rat [789, 478, 1345] and no effect on the number and intensity of histochemical reactions of the EC in the stomach fundus of the rabbit [1345]. Also, reserpine has only a moderate action on the 5-HT content of the gastro-intestinal mucosa of the rat [366, 1300], and no action on the 5-HT in the fundus region of the rabbit stomach [1345].

Finally, reserpine is found to be ineffective both on the 5-HT content [919] and the number of the chromaffin cells of the posterior salivary glands of *Octopus vulgaris* [386].

d) Pre-treatment with iproniazid, a MAO inhibitor, hinders the effect of reserpine both on the 5-HT content and on the intensity of histochemical reactions of the EC in the gastro-intestinal mucosa of the guinea-pig [1346].

e) Argentaffin carcinoids, which are tumours made up of typical EC, contain conspicuous amounts (up to 1–3 mg per g wet tissue) of 5-HT. This substance is lacking in all other epithelial tumours of the intestine, including non-argentaffin carcinoids.

f) Administration of tryptophan by mouth produces an increase in both the number of EC [957], and the 5-HT content of the intestinal mucosa [546]. The opposite has been observed on feeding a low-tryptophan diet [1347].

g) Finally, there have been satisfactory correlations between the chemical colour reactions observed on paper chromatograms of 5-HT pre-treated with formalin with the histochemical reactions shown by the granules of the enterochromaffin cells after formol fixation [367].

Similarly, the enterochromaffin cells and the 5-HT incorporated into gelatin models give nearly identical colour reactions after formol fixation [86].

In view of the above decisive pieces of evidence the opinion of LILLIE *et al.* [740, 494] that mammalian EC do not contain 5-HT but, rather, a phenolic substance, should be withdrawn definitively.

Like catecholamines in the adrenal medulla, and histamine in mast cells, 5-HT is stored in the cells of the gastro-intestinal mucosa in granules distinct from mitochondria [57, 963].

It has been claimed that the number of EC in the rat gastro-intestinal tract decreases following X-radiation [1235], colchicin administration [1256c], hypophysectomy [478c] and during artificial hibernation [1235] or anaemia [1275]. The number of the EC increases remarkably in the duodenum, but not in the ileum of rats deprived operatively of the large intestine [478b].

Besides the typical EC the gastro-intestinal mucosa of vertebrates usually contains other granular cells which are indistinguishable from the EC both in their morphology and in their unspecific staining properties. However they do not display any specific histochemical reaction and have no 5-HT in their granules. For details on these «pre-enterochromaffin argyrophil cells», in part surely belonging to the EC system, we refer the reader to other papers [411, 367, 256].

It has already been stated that 5-hydroxyindole derivatives other than 5-HT may contribute to or even be totally responsible for the positivity of the specific histochemical reactions of the cells belonging to the EC system. This is especially true for the cutaneous glands in amphibian skin.

So far, no histochemical localization of tryptamine and melatonin has been described or reported.

It is to be expected that, even if present in considerable concentrations, these compounds will be difficult to identify with the available histochemical reactions owing to the lack of free phenolic hydroxy groups in their molecules.

The first appearance during embryonic life of EC in the gastro-intestinal tract occurs in the 11th week in man [911], in the 6th to 8th week in oxen [394], on the 16th day in mice [1275], and on the 10th to 16th day in chickens [476, 838b].

3. Occurrence and Distribution of Indolealkylamines in the Living Organism

3.1 Vertebrates

3.11 Gastro-intestinal tract

From the available data it appears clearly that 5-HT is a normal constituent of the gastro-intestinal mucosa of all vertebrates, as well as of ascidians and *Amphioxus*. It is highly probable that all the gastro-intestinal 5-HT, or the greatest part of it, is stored in the enterochromaffin cells.

By administration of radioactive tryptophan and through the study of the daily excretion of 5-HIAA it has been calculated that under normal conditions the half-life of intestinal 5-HT is approximately 4 to 12 hours [368, 1229, 1231]. However when tryptophan is administered in excess, especially by mouth, a

quantity of 5-HT corresponding to that present in the intestines may be produced within 3–4 hours. BERTACCINI [102] has calculated that the rat gastro-intestinal mucosa may synthesize 5-HT, under a tryptophan load, at a rate of 1 $\mu\text{g/g/hour}$.

In the intestine, too, as in platelets and in mast cells, 5-HT is contained in a particular granular fraction which is rich in ATP, the molar ratio 5-HT:ATP being 2.4–3 [963].

The general occurrence of 5-HT within the gastro-intestinal mucosa of all vertebrates represents in the writer's opinion the most convincing proof of the general biological significance of the 5-HT of enterochromaffin origin. It will be seen that, with a few exceptions, the alimentary canal is the only source in vertebrates of the 5-HT found outside the central nervous system.

The figures referring to the 5-HT content in the gastro-intestinal mucosa of common laboratory animals not infrequently show considerable differences according to different investigators. This may depend upon the diet, the bacterial flora of the intestine [1183c, 1274c), and the assay procedure employed.

The small intestine of thiamine deficient rats presents, like blood and spleen, reduced levels of 5-HT [454d).

Table 1
The 5-HT content of some extracerebral tissues of the commonest laboratory animals

Animal species	5-HT content (in μg base per g fresh tissue)				
	Gastro-intestinal tract	Lung	Liver	Kidney	Skin
Dog [906, 364]	2.8–5.2	0.26	0.54	==	0.03
Cat [906, 364]	0.5–1.2	0.62	0.56	=	0.08–0.1
Rabbit [364, 906, 1268, 1270, 1287, 1289b]	2.7–18.0	1.2–4.6	0.27–2.3	0.09	0.04–0.1
Guinea-pig [84, 364, 906, 1268, 1289b]	0.7–7.0	0.06–0.3	0.02	=	0.02–0.18
Rat [99, 145, 364, 901, 906, 1199, 1289b]	1.1–9.0	1.1–3.4	0.14–0.52	0.1–0.15	0.4–4.2
Mouse [336, 364, 906, 1268, 1271, 1289b]	1.6–8.8	1.4–5.2	0.6–1.1	1.3	0.37–1.12
Hamster [906, 364]	..	2.7	0.22	==	0.08
Hen [364]	4.1–5.0	==	==	=	==
Pigeon [364]	1.1	==	==	==	==
Toad [364]	1.6–2.2	==	=	=	==

3.12 Blood

Practically all the 5-HT found in serum or in extracts of whole blood originates from the thrombocytes. Platelet-free plasma contains only traces of 5-HT (0.002 $\mu\text{g/ml}$ [606b] or less than 0.0001 $\mu\text{g/ml}$ [33]), although it should necessarily be admitted that all 5-HT present in the platelets has been absorbed from the plasma and is destined to be discharged into the plasma.

There is at present no doubt that platelet 5-HT is of intestinal origin, with the possible partial exception of the few animal species containing 5-HT in their mast cells.

By the use of radioactive tryptophan it has been calculated that the half-life of platelet 5-HT is 24 to 48 hours, i.e. that the platelets practically lose their 5-HT only when disintegrating [1229, 1231].

Assuming in man a daily production of 5–20 mg 5-HT (as inferred from the urinary excretion of 5-HIAA [368]), and a total 5-HT content in the platelets of 0.5–1 mg 5-HT [1198], it should be concluded that either the turnover rate of 5-HT in the platelets is considerably faster than that calculated or that

Table 2.
The 5-HT content, in μg of the base, in the blood and spleen tissue of some mammalian species

Animal species	5-HT content	
	Blood	Spleen
Man		
whole blood (1 ml)	0.06–0.4 [1264b, 545, 554, 401]	
serum (1 ml)	0.07–0.22 [761, 364, 896, 1356, 1081]	
platelets (10^9)	0.3–0.9 [545, 547, 605, 514, 1079]	
platelet protein (1 mg)	0.17–0.5 [554]	
Rabbit		
whole blood (1 ml)	3.2–5.5 [1268, 1272, 1109]	12–25 [906, 364, 460b]
serum (1 ml)	2.0–4.0 [1247, 856, 364]	
Guinea-pig		
whole blood (1 ml)	0.2 [1268]	1.1–1.6 [364, 906]
serum (1 ml)	0.15–0.70 [761, 364]	
platelet protein (1 mg)	0.3–0.5 [554, 1109]	
Rat		
whole blood (1 ml)	0.35–0.78 [145, 880]	1.5–4.0 [906, 364, 99]
serum (1 ml)	0.3–1.0 [364, 99]	
platelet protein (1 mg)	1.0–2.0 [1109]	
Mouse		
whole blood (1 ml)	3.2–5.5 [1271]	1.8–6.0 [336, 364, 906]
serum (1 ml)	1.5 [364]	
Dog		
serum (1 ml)	0.2–0.3 [213, 364]	1.0–4.6 [906, 364]
Cat		
serum (1 ml)	3.8 [364]	8.5 [906, 364]
Hamster		
serum (1 ml)	0.40 [364]	20.5 [906]
Pig		
whole blood (1 ml)	0.4 [520b]	1.2 [364]
serum (1 ml)	0.25 [364]	
platelets (10^9)	2.2 [520b]	

barely 2.5–20% of the 5-HT produced by the enterochromaffin cells is absorbed by the platelets.

It will be seen later on that the absorption capacity of 5-HT by the thrombocytes is by no means saturated under normal conditions.

For a better understanding of the data shown in Table 2 it should be emphasized that blood serum contains only part (43–59%) of the 5-HT present in the blood [544, 1166]. The remainder is lost during the separation of serum owing to irreversible absorption by red cells [1165] or to enzyme attack.

3.13 Spleen

5-HT is regularly contained, in considerable amounts, in spleen tissue. The substance originates to the greatest extent in the thrombocytes and thrombocyte fragments caught in the spleen. Mast-cell 5-HT contributes scarcely, even in rats and mice, to the total spleen 5-HT. Generally the amine is particularly abundant in spleen tissues of animal species whose blood is rich in 5-HT (e.g. rabbit, ox, hen).

3.14 Mast cells

The occurrence of 5-HT in mast cells was first demonstrated by BENDITT *et al.* [82] in 1955. Since then, considerable work has been dedicated to the study of this peculiar localization of 5-HT in the mammalian organism and some fundamental facts may now be considered as firmly established:

a) Only rat and mouse mast cells contain important amounts of 5-HT. The amine is not detectable in mast cells of other mammalian species, either under normal or pathological conditions [112, 906]. The consequence of this statement is that, from a general biological point of view, 5-HT in mast cells of the above rodents is little more than a biochemical rarity, exactly as is the occurrence of 5-HT in the hypobranchial body of some molluscs or in the coelenteron tissues of some coelenterates.

Mast cells do not necessarily contain the same quantity of 5-HT in the different rat tissues, nor is the 5-HT/histamine ratio everywhere the same [112].

RICE and MITCHENER [980b] have recently claimed to have obtained histochemical evidence of the occurrence of 5-HT in a dog mast cell tumour. Unfortunately, the investigators were unable to support their not completely persuasive histochemical data (variable silver reaction!) with any biochemical or biological evidence.

b) 5-HT in the mast cells is probably of completely autochthonal origin, i.e. it may be synthesized from L-tryptophan within the mast cells themselves. This has been shown by SCHINDLER *et al.* [1084, 1083] and by DAY and GREEN [291], who found that neoplastic mast cells obtained from a mouse mastocytoma after many generations in culture maintained, or even increased, their high intracellular level of 5-HT.

Rat mast cells show considerable 5-HTP-decarboxylase activity [536, 693], but so far tryptophan hydroxylase activity has not been demonstrated [1128].

c) 5-HT is contained, together with heparin and histamine, in specific granules of the mast cells which are clearly separable from mitochondria and other intracellular organelles [535]. It is possible that both histamine and 5-HT are synthesized in the non-particulate cytoplasm of the mast cells and then stored in a biologically inactive form, in the basophilic granules [536b), perhaps in combination with heparin [644]. Since heparin from a mouse mastocytoma and from rat tissues has yielded, on fractionation, an unusual heparin fraction, it has been suggested by GREEN *et al.* [517] that the anomalous occurrence of 5-HT in the mast cells of rats and mice may be explained by the presence of this unique heparin.

Table 3
The 5-HT content of mast cells

Rat	Normal mast cells
	Mast cells from peritoneal cavity: 630–700 μg 5-HT base per ml cells [82]
	Mast cells from peritoneal cavity: 0.346–0.462 μg 5-HT base per 10^6 cells [642]
	Mast cells from feet, areolar tissue: 760 μg 5-HT base per ml cells
	Mast cells from feet, dermis: 780 μg 5-HT base per ml cells
	Mast cells from back, areolar tissue: 800 μg 5-HT base per ml cells
Mouse	Mastocytoma
	Tumoral tissue: 8–714 μg 5-HT base per g tissue [447, 1126, 872]
	Tumoral mast cells in culture: 0.06–1.5 μg 5-HT per 10^6 cells [518, 1083, 1084]
Dog	Mastocytoma
	Tumoral tissue: <0.2 μg 5-HT base per g fresh tissue [906, 1126] 0.32–0.83 μg 5-HT per g lyophilized tissue [819]
Cow	Mastocytoma
	Tumoral tissue: no detectable 5-HT [906]
Man	Mastocytoma: no 5-HT detectable in tumoral tissue [906]
	Urticaria pigmentosa: <0.25 μg 5-HT base per g skin [906, 1126]

The turnover rate of mast-cell 5-HT is not known. Indirect evidence supports the view that under normal conditions it is probably rather slow, but that in the case of mastocytoma it may become more rapid. In fact, BERTACCINI [99] has shown that removal of the entire gastro-intestinal tract in rats causes the practical disappearance of 5-HIAA in urine, while leaving largely unaffected the mast-cellular 5-HT in liver, lung and skin, which accounts for more than 50% of the total 5-HT in the body. On the other hand, SJOERDSMA *et al.* [1126] have found that mouse mastocytoma provokes not only a huge increase in the 5-HT content in the mouse body (from 1.5 to 20 $\mu\text{g/g}$) but also a similar increase in the urinary 5-HIAA (from 6 to 70 $\mu\text{g/ml}$).

LEWIS [734] considers each mast cell of rats and mice as a minute pharmacological armamentarium destined not only to serve as outer defence in the body defence mechanism against tissue injury, but also as a store-house of

vaso-active substances which may well be released physiologically to control the vasculature of the connective tissue.

This view has been recently strenghtened by the observation that mast cells may contain not only histamine and 5-HT but, at least in ungulates, also dopamine [281b].

3.15 Other extracerebral tissues

The few animal species (practically only rats and mice) containing 5-HT in their mast cells display considerable 5-HT levels in nearly all their parenchymatous tissues. However a correlation between mast-cell population and 5-HT concentration is not always evident. PARRATT and WEST [903, 906], for example, maintain that the abundant 5-HT which is found in the outer layer of the rat skin is not held in mast cells. This opinion is shared by SMITH and LEWIS [1142b) on the basis of results obtained in rats with an anti-mast cell serum, which caused no fall of skin 5-HT at the time when histamine and mast cells in the skin were very low. Similarly, a mast-cell origin is doubtful for the 5-HT found in the thyroid gland of the rat.

Concerning unusual localizations of 5-HT in other animal species, two seem of particular interest: that in the rabbit lung and that in the sheep thyroid gland. The 5-HT in the rabbit lung clearly originates from blood platelets, as shown by the fact that during anaphylactic shock, in which circulating platelets are caught in the lung, lung 5-HT may increase by 5 to 10 times [1268]. The origin of the 5-HT contained in the sheep thyroid gland is unknown. It would seem advisable to find out whether in some species enterochromaffin cells are contained in thyroid tissue as they are in thymus tissue.

No significant changes in the 5-HT content of the rat thyroid gland are seen following single doses of methimazole or potassium thiocyanate. Repeated administration of methimazole, however, produces a significant increase of the total 5-HT in the gland [885].

Minute amounts of 5-HT have been described in a variety of tissues of different species, but very often one cannot avoid the impression that this 5-HT

Table 4
The 5-HT content of the whole brain of some vertebrates

Animal species	μg 5-HT base per g fresh tissue	Animal species	μg 5-HT base per g fresh tissue
Rabbit	0.45-0.57 [167, 524, 934]	Turkey	0.4
Guinea-pig	0.58-0.68 [554, 936]	Lizard	0.1
Rat	0.21-0.96 [145, 879, 500, 1233, 935]	Tortoise	0.1 ✓
Mouse	0.66-1.0 [340, 1113, 935, 15]	Water snake	0.2
Pigeon	0.15 [271]	<i>Scylliorhinus</i>	
Hen	0.2 [271]	<i>canicula</i>	0.2 [271]

may be referred to the blood contained in the tissues [460b]; or that colour reactions, fluorescence reactions or pharmacological actions considered specific for 5-HT may in reality depend on substances other than 5-HT.

Several parenchymatous tissues (lung, liver, kidney) contain apparently small amounts (0.05–0.2 $\mu\text{g/g}$) of tryptamine [574]. This is all we know at present about the occurrence and distribution of this amine in the mammalian organism.

Table 5
Regional distribution of 5-HT in the brain of the dog, cat and rabbit

	5-HT content, in μg base per g fresh tissue		
	Dog	Cat	Rabbit
Brain stem	0.75 [175]	0.79 [175]	0.45–0.57 [175]
Medulla	0.55–0.62 [140,139]	0.55–1.2 [140,139,684b]	0.63 [276]
Area postrema	0.26 [876,877]		
Floor 4th ventricle	0.23		
Pons	0.38–0.42	0.33–0.7	
Pontile reticular formation		0.89	
Cerebellum	0.07; 0.01	0.27–0.30	0.12; 0.03 [460b]
Midbrain	0.97	1.23–1.7	0.83; 0.31
Midbrain reticular formation		2.6	
Inferior colliculus		0.76	
Thalamus	0.57–0.65	0.43–1.1	
Hypothalamus	1.75; 0.37	1.78–2.5	0.39
Olfactory bulb	0.38–0.35; 0.08		
Intralaminar and midline nuclei		0.56	
Brain gyri	0.17	0.24	
White matter	0.07–0.13; 0.02	0.13	
Cortical gray matter	0.34–0.27	0.68	
Amygdala	2.1; 0.49	1.6	
Caudate nucleus	0.72; 0.27	1.6	
Pyriform cortex	0.94; 0.33	1.4	
Auditory cortex		0.41	
Cingulate cortex		0.41	
Central grey matter		1.6	
Hypnogenic zone of Hess		1.8	
Hippocampus	0.64; 0.26		
Septal region	1.5; 0.46	2.0	
Sympathetic ganglia	0		
Posterior pituitary	0		

3.16 Central and peripheral nervous system

The occurrence of 5-HT in the CNS was first demonstrated by AMIN *et al.* [26] and by TWAROG and PAGE [1217b] and soon confirmed by numerous other research workers. It may be seen from Tables 4, 5 and 6 that all vertebrate species examined contain 5-HT and/or strictly related substances in the brain. This fact represents again, as in the case of gastro-intestinal mucosa, strong evidence that 5-HT in the brain has a general biological significance.

Table 6
Regional distribution of 5-HT in the human brain [274, 104b]

Brain area	5-HT content (μ g base per g fresh tissue)	Brain area	5-HT content (μ g base per g fresh tissue)
<i>Isocortex</i>		Nucleus caudatus	0.20–0.70
Frontal cortex	0.01–0.04	Fornix (anterior pars)	0.22–0.55
Gray matter of frontal cortex	0.03–0.08	Corpus callosum	0.20–0.39
Frontal pole	0.01–0.03	Amygdaloid nucleus	0.24–0.67
Temporal pole	0.01–0.07	Thalamus	0.23–2.70
Occipital pole	0.01–0.06	Hypothalamus	0.32–1.53
<i>Allocortex</i>		Mammillary bodies	0.27–0.48
Cingulate gyrus	0.04–0.10	Anterior quadr. bodies	0.50–1.22
Hippocampal gyrus	0.10–0.30	Posterior quadr. bodies	0.29–0.81
Uncus	0.16–0.30	Substantia nigra	1.11–1.96
Sub-callosum gyrum	0.14–0.60	Pons	0.19–1.03
Anterior perforated substance	0.41–1.51	Medulla oblongata	0.20–0.50
Hippocampus	0.06–0.19	Area postrema	0.42–0.81
<i>Neostriatum</i>		Cerebellum	0.01–0.09
Putamen	0.23–0.95	Cervical spinal cord	0.09–0.20
		Liquor [37, 398, 1217]	<0.001–0.01

Tables 5 and 6 show that, at least in mammals, 5-HT is preferentially contained in specific areas of the brain (brain stem structures, rhinencephalic structures, neostriatum) some of which are part of, or are functionally connected with, the autonomic system or with the reticular activating system.

Although the problem of the origin of cerebral 5-HT is not completely solved, indirect evidence seems to support the view that even brain 5-HT may originate locally from L-tryptophan. We refer to the experiments of BERTACCINI [99] showing that 5-HT is present in nearly normal amounts in the brain of rats deprived operatively of the whole gastro-intestinal tract, and to the occurrence of considerable quantities of 5-HT in ganglia and peripheral nerves of molluscs containing only traces of the amine in all other tissues.

On the other hand, if brain tissue is known to be capable of decarboxylating 5-HTP, there is so far no direct evidence that it is capable of hydroxylating L-tryptophan.

The turnover rate of 5-HT in the central nervous system is high. With the aid of powerful, promptly-acting aminoxidase inhibitors it has been demonstrated that the half-life of cerebral 5-HT is not longer than 10 to 20 minutes [1229, 1231] and according to 5-HT estimates in brains of rats anaesthetized with pentobarbital sodium it is even shorter (1 to 2 minutes) [16b].

Concerning the problem of the distribution of 5-HT at cellular and subcellular levels within the brain tissue, there is still considerable uncertainty and perplexity. Three main possibilities should be kept in mind, namely that:

a) 5-HT is contained, at least in some areas, in glia cells instead of in true nervous cells. This possibility is supported by the finding that in autoradiographs of brain tissue obtained after injection of radioactive 5-HTP, the 5-HT appears to be distributed fairly evenly throughout the brain with no relation to nervous elements [735]; and by the observation that in the dog whole CNS the highest concentrations of 5-HT are found in the area postrema which consists of highly vascular neuroglia tissue and only some nervous cells [1168].

b) 5-HT is present in the body of the nervous cell. This is the opinion shared, tacitly or expressly, by the majority of research workers. According to WALASZEK and ABOOD [1273] most of the 5-HT present in rat brain is bound to the mitochondria but other investigators [500, 481], while confirming that 70–80% of the brain 5-HT resides in granules, eschew a more precise definition of the nature of these granules.

c) 5-HT is contained in the nerve endings, more precisely in the synaptic vesicles or in particles quite similar to them. This theory, which has been advanced by WHITTAKER [1311, 1312, 1313], is at present the most satisfactory and attractive. WHITTAKER suggests that, like acetylcholine, 5-HT also may be bound to the particles partly in a highly labile state and partly in a more stable state. Subcellular particles containing 5-HT should be different from those storing acetylcholine.

It has been further observed by the same investigator [1312b] that 5-HT can be taken up from the supernatant by the particles, and that this process is ATP dependent. Reserpine does not appear to affect 5-HT uptake nor does it deplete the particles of 5-HT *in vitro*, although it is possible to prepare depleted particles from animals previously treated with reserpine *in vivo*.

ALBRECHT *et al.* [15] found that the brain 5-HT level is slightly higher in female rats than in male adult mice (0.93 μg versus 0.78 $\mu\text{g/g}$) and that a small but significant decrease in 5-HT concentration of brain pools is noted prior to the usual time of arousal. On the average the concentration of 5-HT in brain is higher before and around noon than at or after 4.30 p.m. (difference 0.12 $\mu\text{g/g}$).

TOH [1199] observed that the 5-HT content of the brains of rats exposed to extreme heat or cold is lower (0.20 and 0.24 $\mu\text{g/g}$) than that of control rats (0.38 $\mu\text{g/g}$).

There are only few and incomplete data on the appearance of brain 5-HT during embryonic life. KÄRKI *et al.* [632] and NACHMIAS [855b] found that three days before birth rat brain 5-HT is about 25% (norepinephrine 15%);

at birth 50% (norepinephrine 20%); and two weeks later 70% (norepinephrine 40%) of normal, with values of the mature animals being approached in seven weeks. In contrast to rats, guinea-pig at birth have brain levels of both 5-HT and norepinephrine as high as those found in mature animals.

It has been suggested that the above biochemical differences should be correlated with differences in development of the behaviour pattern in the two species. However, this hypothesis seems to be premature since in the goat foetus, late in gestation, the 5-HT content of the parietal lobe is much higher than that observed in maternal parietal lobes (0.37 versus 0.11 $\mu\text{g/g}$) [914b].

Besides 5-HT, tryptamine also should be considered as a normal constituent of brain tissue. The brain content of this amine seems, however, to be rather low (0.1 $\mu\text{g/g}$) [574] and information about regional differences in its distribution is lacking.

Among the areas of the CNS, the pineal gland occupies a particular position since, in addition to high amounts of 5-HT it contains two unusual 5-hydroxy-indole derivatives: melatonin and its metabolite 5-methoxyindoleacetic acid [723]. The 5-HT in the pineal gland shows surprisingly remarkable individual and species differences, a circumstance which casts some doubt on the importance of the function of 5-HT in this localization (precursor of melatonin?). However, as pointed out by GIARMAN and FREEDMAN [483], the 5-HT levels found in the majority of human and simian glands are the highest ever reported in any neuronal structure of all species examined.

DE IRALDI and DE ROBERTIS [308c] suggest that 5-HT is contained, together with other biogenic amines, in the secretory processes of the pinealocyte, and more precisely in the heterogenous vesicles of these processes. This suggestion seems to be supported by the disappearance of the heterogeneous vesicles following reserpine administration.

Table 7
Indole derivatives in the pineal gland

	Content in μg per g fresh tissue			
	5-HT	Melatonin	5-HIAA	5-Methoxy IAA
Steer	0.4	0.04–0.06	+	0.04–0.06 [721, 723, 481, 483]
Monkey	1.2–10			[482]
Man	0.4–23			[482]

The problem of whether autonomic ganglia of mammals contain 5-HT and are capable of synthesizing the substance cannot be considered solved by the partially positive results of GERTNER *et al.* [471, 472], which contrast with the negative results of others [26].

Peripheral nerves apparently do not contain 5-HT. However a melatonin-like substance has been described in some of them [722].

3.17 Amphibian skin

Amphibian skin presents one of the most conspicuous localization of indolealkylamines in the living organism. No tissue can compete with the parotoid glands of some toads as regards the absolute values of indolealkylamines, and in no tissue is there such a variety of indolealkylamines. It is therefore conceivable that comparative studies on the skin of adult and larval amphibians may contribute substantially to a better understanding of the metabolic possibilities of these substances in the living organism. Table 8 shows the indolealkylamine content in the skin of some representative amphibians [364].

In all probability indolealkylamines in this localization are of completely autochthonal origin i.e., they are fully independent, in regard to biosynthesis, of gastro-intestinal mucosa.

Table 8
The occurrence of indolealkylamines in the skin of some amphibians [364]

	Tryptamine	5-HT	N-Methyl 5-HT	Bufotenine	Bufotenidine	Bufothionine	Dehydro- bufotenine	Bufoviridine
<i>Salamandra maculosa</i>	+	+	0	0	0	0	0	0
<i>Triton cristatus</i>	+	0	0	0	0	0	0	0
<i>Xenopus laevis</i>	0	+	0	0	+++	0	0	0
<i>Discoglossus pictus</i>	0	+++ (400 µg)	0	0	0	0	0	0
<i>Bufo bufo bufo</i>	0	+	+	++	+	0	+	0
<i>Bufo viridis</i>	0	+	+	+++ (640 µg)	+	0	+	+++ (430 µg)
<i>Bufo americanus</i>	0	++	+	++	++	0	+	0
<i>Bufo fowleri</i>	0	++	+	++	++	0	+	0
<i>Bufo arenarum</i>	0	+	?	++	+	++	++	0
<i>Bufo mauretanicus</i>	0	+++	?	0	0	0	0	0
<i>Bombinator pachypus</i>	0	+++ (1000 µg)	0	0	0	0	0	0
<i>Hyla arborea</i>	0	++	0	0	0	0	0	0
<i>Rana esculenta</i>	0	+	0	0	0	0	0	0
<i>Rana pipiens</i>	0	+	0	0	0	0	0	0
In parentheses some amine values per g fresh tissue								

Indolealkylamines in the skin of amphibians are destined exclusively for external secretion and their biosynthesis is therefore strictly connected with the biosynthesis of the other constituents of the «cutaneous venom». This suggests that, in spite of all appearances, the turnover rate of the amines in the skin must be rather slow.

The biological significance of the indolealkylamines in cutaneous venom is largely obscure. It may be that they play some role in the defence of the animal, but is also possible that they simply constitute metabolic end products.

3.18 Male reproductive tract of the dogfish

The undiluted secretion of clasper siphons of sexually mature specimens of *Squalus acanthias* (250 mg of fluid per pair of clasper siphons) contains, according to MANN [772b], the tremendous amount of 25–34 mg 5-HT base per g secretion; the siphon tissue itself (2.8–3.6 g per kg body weight) 1.2 mg 5-HT per g wet tissue. This would correspond to no less than 10 mg 5-HT per animal weighing 1 kg. 5-HT was absent or present only in traces in the sperm removed directly from the seminal ducts and in the so-called seminal vesicles. Clasper siphons of immature males, weighing 100 mg, contained only 0.07–0.20 mg 5-HT per g tissue.

It has been suggested that the 5-HT present in a secretion which represents an integral part of semen might play a part in the reproductive process, either by affecting the mechanism of copulation and ejaculation in the male, or by eliciting contractions of the female reproductive tract, thus influencing passage of sperm and fertilization.

3.19 Venom of reptiles

ZARAFONETIS and KALAS [1344c] have recently described the presence of very minute amounts of 5-HT in the venom of certain reptiles: *Heloderma horridum*, 5.2 µg 5-HT creatinine sulphate/ml venom; *Crotalus atrox*, 0.32–0.78 µg/ml; *Crotalus adamanteus*, 0.24 µg/ml; *Agkistrodon piscivorus*, 0.84 µg/ml. 5-HT was accompanied by other known (tryptamine, 5-HIAA, IAA) and unknown indole compounds.

It seems futile to ascribe to these unimportant amounts of indolealkylamines any significance in the complex action of the snake venom.

The occurrence of 5-HT in the reptilian venom does not seem to be constant. In fact, WELSH [1298c] failed to detect the amine in the venom obtained from different reptilian species.

3.2 Invertebrates

So far 5-HT has been detected in particularly high amounts in some venom apparatuses of molluscs, scorpions, insects and coelenterates (Table 9) and in much less conspicuous but important amounts in the nervous tissue of molluscs (Table 10).

It is certain that numerous other examples of indolealkylamines will be discovered in invertebrates.

The 5-HT in the venom glands does not seem to have any particular importance but that of a facultative constituent of the venom. In fact the venom of strictly related species may present, for completely unknown reasons, enormous differences in 5-HT content. This is true for the posterior salivary glands and for the hypobranchial body of molluscs, for the venom apparatus of *Hymeno-*

Table 9

Occurrence of 5-HT and related indole derivatives in invertebrate tissues other than nervous tissue

Animal species and tissue		5-HT	Other indole derivatives
Molluscs	Posterior salivary glands		
	<i>Octopus vulgaris</i> (Bari)	300–500 µg	[364]
	<i>Octopus vulgaris</i> (Bermudas)	68–72 µg	[1298b]
	<i>Eledone moschata</i>	400–750 µg	[364]
	<i>Octopus macropus</i>	<0.5 µg	[386b]
	Median salivary gland		
	<i>Loligo pealii</i>	0.38 µg	[1298b]
	Anterior salivary glands		
	<i>Octopus vulgaris</i> (Bermudas)	0.48–2.4 µg	[1298b]
	Tissues from diff. mollusc. spec.		
	Intestine	0.6–0.7 µg	
	Gill	0.1–1.0 µg	
	Heart	0.2–0.5 µg	
	Kidney	0.26–2.0 µg	
	Musculature	0.08–1.0 µg	
Crustaceans	Connectives	2.2 µg	
	Skin	<0.04 µg	[510, 1218, 1297, 1298b]
Crustaceans	Green glands	0.06–1.0 µg	[1298b]
	Pericardial organ	0.3–4.0 µg	Ortho-dihydroxytryptamine [226, 1298b]
Insects	Wasp venom apparatus	0.06–0.8 µg per apparatus	[364]
	Hornet venom apparatus	7.5–19 µg p. mg dry ven. sac	[115]
	Honeybee venom apparatus	0.0005; 0.03 µg per appar.	[615 b, 364, 1298b]
Scorpions	<i>Leiurus quinquestriatus</i>	2–4 µg per mg dry venom	[6]
	<i>Buthotus minax</i>	0.03–0.04 µg p. mg dry ven.	
	<i>Vejovis</i> sp.	50–138 µg per sting segment	[1298b]
	<i>Hadrurus arizonensis</i>	<0.55 µg per sting segment	
Worms	<i>Dugesia tigrina</i> whole	2.0 µg	
	<i>Dugesia dorotocephala</i> whole	1.5–3.4 µg	
	<i>Lineus ruber</i> whole	0.18–0.43 µg	
	<i>Cerebratulus lacteus</i> whole	0.08–2.9 µg	
	<i>Arenicola marina</i> body wall	2.3 µg	
	<i>Amphitrite ornata</i> gills	0.26 µg	
	body wall	0.19 µg	[1298b]
Coelenterates	<i>Fasciola hepatica</i>	0.06–0.07 µg	[775]
	<i>Hydra oligactis</i> whole	1.5 µg	[1298, 1298b]
	<i>Calliactis parasitica</i>		
	coelenteric tissue	500–660 µg per g	
	column	15–36 µg freeze-dried	
	tentacles	6–12 µg tissue	[797, 798]
	<i>Metridium senile</i>		
	whole	0.04–0.05 µg	
Coelenterates	tentacles	0.24–0.47 µg	Bufotenine, 5-HTP
	acontia	0.60–1.3 µg	[917, 1298, 1298b]

Unless otherwise stated the above values are per g fresh tissue

Table 10
Occurrence of 5-HT in nervous tissue of invertebrates

Animal species		Tissue	5-HT content (in μg base per g fresh tissue)
Molluscs	<i>Venus mercenaria</i>	pooled ganglia	30-40
	<i>Pecten magellanicus</i>	visceral ganglia	36
	<i>Mytilus edulis</i>	ganglia	10-15
	<i>Artica islandica</i>	ganglia	20
	<i>Spisula solidissima</i>	ganglia	8-14.3
	<i>Ensis directus</i>	ganglia	21-39
	<i>Mya arenaria</i>	ganglia	22
	<i>Buccinum undatum</i>	ganglia	7.7
	<i>Busycon canaliculatum</i>	ganglia	8.4-9.7
		nerves	2-2.5
	<i>Fasciolaria tulipa</i>	ganglia and nerves	7.6-9.4
	<i>Loligo pealii</i>	«brain»	0.7
		optic ganglia	1.15
	<i>Octopus briareus</i>	pooled ganglia	1.6 [1297, 1298b]
	<i>Octopus vulgaris</i>	«brain»	0.8; 0.25
		optic ganglia	2.3; 2.3 [1298b, 386b]
		stellate ganglia	0.72
		nerves	1.5 [386d]
	<i>Eledone moschata</i>	optic ganglia	2-4
		nerves	1.5-2 [98, 102]
Crustaceans	<i>Cancer irroratus</i> , <i>Cancer borealis</i> , <i>Carcinus maenas</i> , <i>Homarus americanus</i> , <i>Panulirus argus</i> , <i>Orconectes virilis</i> , <i>Palimnurus vulgaris</i>	{ nerve cords, ventral ganglia, «brain», leg nerves }	<0.1 [368d, 1298b]
Worms	<i>Lumbricus terrestris</i>	nerve cords	10.4 [1298b]
	<i>Hirudo medicinalis</i>	nerve cords	6.9; <0.2 [1298b, 386d]
	<i>Amphitrite ornata</i>	nerve cords	5.4
	<i>Arenicola marina</i>	nerve cords	3.1
	<i>Glycera dibranchiata</i>	nerve cords	4.6 [1298b]
Insects	<i>Blaberus gigantea</i>	nerve cords	<0.02 [1298b]
	<i>Locusta migratoria</i>	ganglia	<0.2 [386d]

ptera, for the venom apparatus of scorpions, etc. It cannot be excluded that, as for amphibian skin, 5-HT is here merely a metabolite destined for external secretion.

Things are completely different for the 5-HT present in molluscan nervous tissue. All the examined molluscs, without exception, contain 5-HT in their ganglia and peripheral nerves, and this 5-HT behaves, towards different drugs, exactly as the 5-HT in the brain of mammals. Moreover, the turnover rate of

Table 11
Occurrence of 5-HT and related indole derivatives in plants

Plant species	5-HT	Other indole derivatives
<i>Neurospora crassa</i> [1104]	present	β -hydroxy-pseudo-tryptophan
<i>Panaeolus campanulatus</i> [1222]		other indole derivatives
<i>Panaeolus sphinctrinus</i> [310b]		psilocybin 1.9 $\mu\text{g/g}$
<i>Psilocybe</i> (several species) [584, 585, 310b]		psilocybin 0.1–6 $\mu\text{g/g}$
<i>Stropharia cubensis</i> [310b]		psilocin 0.0–2.5 $\mu\text{g/g}$
<i>Amanita mappa</i> [1313b]		psilocybin 0.1–5 $\mu\text{g/g}$
<i>Piptadenia peregrina</i> (seeds) [1179]		psilocin 0.0–2.5 $\mu\text{g/g}$
<i>Piptadenia colubrina</i> (seeds) [886]		bufotenine
<i>Piptadenia macrocarpa</i> [424]		bufotenine 9.4 $\mu\text{g/g}$; 5-HIAA
		bufotenine
<i>Gossypium hirsutum</i> (fruit) [184]	present	bufotenine, bufotenine oxyde, N,N-dimethyltryptamine, N,N-dimethyltryptamine oxyde
<i>Symplocarpus foetidus</i> (leaves) [184]	present	
<i>Citrullus vulgaris</i> [291b]	present	
<i>Dictyoloma incanescens</i> (bark) [886]		N,N-dimethyl-5-methoxytryptamine
<i>Mimosa hostilis</i> (roots) [886]		N,N-dimethyltryptamine
<i>Urtica dioica</i> (stings) [266]	3–5 $\mu\text{g}/1000$ stings	
<i>Laportea moroides</i> (hairs) [986]	1 $\mu\text{g}/1000$ hairs	
<i>Mucuna pruriens</i> (trichomes) [154b]	150 $\mu\text{g/g}$ trichomes	
<i>Prestonia amazonica</i> (leaves) [583]		N,N-dimethyltryptamine
<i>Acacia fluribunda</i> (tops) [1310b]		tryptamine
<i>Phalaris arundinacea</i> [1315b]		5-methoxy-N-methyltryptamine
<i>Acer saccharinum</i> [886]		gramine
<i>Acer rubrum</i> (fruit) [887]		gramine
<i>Ananas comosus</i> [180, 435, 1304b]	<1.5; 12–25 $\mu\text{g/ml}$ juice	
<i>Carica papaya</i> (fruit) [435]	1.1–2.1 $\mu\text{g/g}$	
<i>Passiflora foetida</i> (fruit) [435]	1.4–3.5 $\mu\text{g/g}$	
Banana (peel) [1232, 1266, 1301]	40–150 $\mu\text{g/g}$	
(pulp)	19–28 $\mu\text{g/g}$	
Plantain (peel) [791, 1232]	1–41 $\mu\text{g/g}$	
(pulp)	19–28 $\mu\text{g/g}$	
Tomatoes (plant) [1302, 1303]	0–0.5 $\mu\text{g/g}$	
(fruit)	1.5–12 $\mu\text{g/g}$	tryptamine 1.8–4 $\mu\text{g/g}$
Eggplant	2 $\mu\text{g/g}$	tryptamine 0.5–3 $\mu\text{g/g}$
Avocado	10 $\mu\text{g/g}$	
Red plum	10 $\mu\text{g/g}$	tryptamine 0.2 $\mu\text{g/g}$
Blue-red plum [1232]	8 $\mu\text{g/g}$	tryptamine 2 $\mu\text{g/g}$

5-HT in the ganglia of molluscs is very high, the half-life of the amine being not longer than 15 to 20 minutes [98].

From this it may be reasonably inferred that, in sharp contrast to other localizations of 5-HT in the molluscan body, neuronal 5-HT must have a general biological significance, probably in the transmission of nervous impulses. Appropriate procedure indicates that 5-HT is contained in the granules in molluscan ganglia [1294].

It is conceivable that a thorough study of the 5-HT in the nervous tissue of molluscs may offer a key to a better understanding of the function of the amine in the brain of vertebrates.

3.3 Plants

The data reported in Table 11 are intended as a mere exemplification of the wide distribution of indolealkylamines in the vegetable kingdom. It may be anticipated that these compounds will be met in numerous other plant tissues.

It is of conspicuous interest that in addition to 5-hydroxyindoles, 4-hydroxyindoles also have been found in the vegetable kingdom. This means that the plant cells contain enzymes capable of hydroxylating the indole nucleus at the 4-position also.

The significance of indolealkylamines in plants is poorly understood. It may be that in some cases they act as growth factors or as precursors of auxines, and that in other cases they intervene in the synthesis of pigments. But a third, good possibility is that, as in the animal kingdom, some examples of indolealkylamines in plants are to be considered as mere end products of their metabolism and with no particular significance.

4. Physiological Uptake, Release and Transport of 5-Hydroxytryptamine

4.1 Physiological release of 5-HT from the gastro-intestinal mucosa

Leaving aside the possible interference of ATP, nothing is known about the nature of 5-HT binding in the granules of the EC [607d] and very little about the physiological mechanisms which regulate the release of 5-HT from the gastro-intestinal mucosa.

It has been claimed that elevation of the intraluminal pressure and/or motility of the intestines produces an increase of the 5-HT released both into the intestinal lumen [187, 188] and into the venous blood [633, 6b]. Although from a theoretical point of view this is quite possible (the chromaffin cells of the posterior salivary glands of octopods, for example, possess both an internal and an external secretion), there is as yet no decisive evidence that EC discharge, under strict physiological conditions, part of their 5-HT into the intestinal lumen. It is, on the other hand, firmly established that the EC can discharge their specific amine into the blood stream [368, 99, 1203b].

GORDON [509b] affirms that following a bleeding of 10 ml/kg, whole blood 5-HT increases in rats and rabbits by about 50% and remains elevated for at least one week. Whether bleeding stimulates by some mechanism release or biosynthesis of 5-HT by the intestinal mucosa remains to be investigated.

In recent experiments, ERSPAMER and BERTACCINI [386c] have found that single or repeated oral doses of water (5 ml/100 g) produce in rats a conspicuous increase (100–300%) of the urinary excretion of 5-HIAA. A similar, or even more striking increase (up to 300–400%) may be obtained with oral or subcutaneous administration of isotonic NaCl solution, as well as with single or repeated water loads plus single or repeated parenteral doses of posthypophyseal antidiuretic hormone. Very frequently, but not necessarily, increased urinary excretion of 5-HIAA coincides with increased diuresis.

Research in progress is intended to elucidate the mechanism by which administration of water or physiological saline produces excess urinary output of 5-HIAA. It is possible, in fact, that at least two factors play a role in the production of the above phenomenon: increased biosynthesis and/or release of 5-HT by the gastrointestinal mucosa and possibly other tissues, and decreased tubular reabsorption of the excreted 5-HIAA.

Preliminary quantitative estimations of 5-HT in the gastrointestinal tract have shown that the small intestine of rats given repeated oral doses of physiological saline or tap water plus antidiuretic hormone contains approximately 30% less 5-HT than the small intestine of control rats.

Rats given by stomach tube 4 ml/100 g paraffin oil, instead of tap water, present no change in the urinary 5-HIAA excretion, thus suggesting that distension of the intestinal wall and hyperperistalsis produced by a non-absorbable, pharmacologically inactive material, does not cause any appreciable release of 5-HT into the blood stream.

4.2 Physiological uptake and release of 5-HT by blood platelets

Apparently only a small percentage of the 5-HT produced by the EC and discharged by them into the blood stream is taken up by the platelets. This casts some doubt on the alleged function of platelets serving as circulating store-houses for 5-HT.

There are conspicuous species differences in the uptake capacity of 5-HT by the platelets and under normal conditions it seems that this capacity is saturated only to a small extent. In fact, the maximum uptake of 5-HT observed by BORN and GILLSON [148e] for human platelets was 1.08 μg per 10^8 platelets, the normal value being 0.025–0.038 μg .

According to MITCHELL and CASS [835b] and GRASSO *et al.* [514], human platelets at birth contain only a small amount of 5-HT because their capacity for taking it up is poorly developed.

Platelets absorb 5-HT even when the concentrations of the amine in plasma are less than 0.002 $\mu\text{g}/\text{ml}$, and the rate of uptake increases with increas-

ing concentrations of 5-HT in the plasma up to concentrations of about 0.5 $\mu\text{g/ml}$ [148d]. The relation between the initial rate of uptake and the external concentration of 5-HT can be described by the Michaelis-Menten equation. *In vitro* the uptake is complete within 60–180 minutes [148b].

At 0–2° the uptake of 5-HT by human platelets is 27 to 42 times less than at 37° [148d]. Sodium cyanide, sodium monoiodacetate [148b], DNP [1044b], fluoride [1291b], 2,4-dinitrophenol in high concentrations [148e], reserpine, cardiac glycosides [1291b] and imipramine [792] inhibit the absorption of 5-HT, whereas potassium [148e, 1291b], 2,4-dinitrophenol in low concentrations [148e], phosphate [1044b, 1291b, 1393], glucose and ATP [1044b] enhance 5-HT accumulation.

Prior mixing of 5-HT with Mg^{++} followed by chelation with sodium EDTA markedly diminishes uptake of 5-HT by intact human platelets in the presence of plasma. Disodium EDTA is without effect, and so Mg^{++} alone. KERBY and TAYLOR [647b] suggest that in the presence of a factor occurring in human plasma, 5-HT is taken up by human platelets as a complex with a divalent cation bound to a heat-stable, non-dialyzable plasma factor.

Whereas all research workers agree that the uptake of 5-HT by the platelets is an active process, there is no concordance of opinion concerning the sources of the energy involved in the process.

BORN *et al.* [50c] believe that one of the factors interfering with the accumulation of 5-HT is ATP and consider as strong evidence for their assumption the fact that in normal platelets a proportion is found between the amount of ATP and the amount of 5-HT both before (molecular ratio $\text{ATP}/5\text{-HT} = 12.8$) and after incubating them in plasma to which 5-HT has been added (ratio = 3.2). Moreover BAKER *et al.* [47b] and PRUSOFF demonstrated that most of the 5-HT in platelets is concentrated in cytoplasmatic granules in which it is associated with ATP. 5-HT is believed to be bound to ATP by ionic bonds.

SANO *et al.* [1044b] suggest that glucose oxidation may supply the energy required for 5-HT uptake, but this opinion is not shared by others. Since the active transfer of 5-HT occurs readily anaerobically as well as aerobically and is not inhibited by 2,4-dinitrophenol, HUGHES and BRODIE [607e], as well as WEISSBACH *et al.* [1291b, 1291c] believe that glycolysis supplies the energy necessary for the process, with the simultaneous oxidative formation of ATP not being a requirement for the uptake of 5-HT *in vitro*.

Once absorbed, 5-HT is retained by the platelets so firmly (half life of platelet 5-HT = 24–48 hours) that it has been suggested that under normal conditions the release of 5-HT *in vivo* may very well be due to the destruction of platelets. Although this may be substantially true, it is necessary to admit that a portion of the platelet 5-HT must be liberated with considerably greater ease and rapidity. This assumption is based on the fact that blood returning from the intestines contains more 5-HT than blood coming from other vascular areas [1203b, 368]. Thus, in the general circulation platelets quickly lose part of the 5-HT they have quickly absorbed from the capillaries of the intestinal wall.

It may be that platelets contain at least two binding sites for 5-HT [172] or, in other words, two or more fractions of bound 5-HT, a labile fraction and a more stable fraction.

Very little is known about the physiological factors which liberate 5-HT from intact platelets. TOH [1203] found a substance capable of releasing 5-HT from platelets in whole blood, or from suspensions of isolated platelets, present in alkaline extracts of the kidney and of the stomach mucosa and submucosa. The releasing action of the liberator, called «nephrosin» was independent of the presence of Ca^{++} ions, and was not mediated by thromboplastin or thrombin. It was inhibited by versene, oxalate and citrate. Nephrosin liberated 5-HT from spleen tissue also, but not from the gastro-intestinal tract.

The results of TOH were confirmed by GIARMAN and POTTER [480b], who similarly observed that extracts from a number of rat tissues were active in releasing 5-HT from mast cells obtained from a mouse mastocytoma, the highest activity being possessed by brain and kidney extracts. The value of further studies on possible physiological liberators of 5-HT is obvious.

It has been known for a long time that 5-HT is released from platelets during blood coagulation. However the exact mechanism of this 5-HT leakage is not understood completely. Thrombin possibly plays a role [1358, 454c] but it seems that ATP breakdown, which occurs rapidly during clotting, is a factor of greater importance [148f, 1166].

Obviously all other factors or agents which cause agglutination, disruption or damage of the platelets can set free a portion of their 5-HT. This has been observed:

- a) following corpuscular blood trauma occurring in extracorporeal circulatory systems (5-HT is released together with ATP) [1053];
- b) upon intravenous injections of crude *E. coli* endotoxin, producing an abrupt fall of both circulating platelets and serum 5-HT, with concomitant small increases in plasma 5-HT [33, 34, 303b, 995b, 303c];
- c) on contact of platelets, *in vitro*, with haemolytic agents (desoxycholic acid, digitonin, lysocytin), bee venom, bacterial toxins [527b] and phenylmercuric hydroxyde [148f] and finally;
- d) on antigen-antibody reaction, both *in vitro* and *in vivo* [1264]. As a possible mechanism for the 5-HT (and histamine) release *in vitro*, as caused by the addition of antigen to platelets suspended in heparinized plasma containing the antibody, HUMPHREY and JAGUES [606] suggest an activation of a protease in plasma by the antigen-antibody reaction.

There is some similarity between what has just been described and the release of 5-HT and histamine from the platelets following incubation of normal rabbit blood with glycogen [1270].

A unique position among the 5-HT releasers is occupied by reserpine and allied tranquillizing drugs. The action of reserpine will be discussed in detail elsewhere. Here we wish only to lay emphasis on the fact that release of 5-HT, and inhibition of 5-HT uptake by reserpine is a general phenomenon for platelets,

subject to no exceptions, and that platelets are an excellent model for studying the mechanism of 5-HT release by reserpine.

Absorption by the platelets of indolealkylamines other than 5-HT has never been demonstrated either *in vivo* or *in vitro*.

Similarly, it is not known whether 5-HTP can be taken up by platelets. This uptake—which does not seem probable from the experiments of SCHANBERG and GIARMAN [1060] showing that spleen takes up 5-HTP only by diffusion—would offer an acceptable explanation for the *in vivo* release of histamine from rabbit platelets produced by intravenous injection of 50–100 mg/kg 5-HTP [193].

4.3 Binding of 5-HT and release from mast cells

As in the case of EC very little is known about the nature of the 5-HT binding in the mast cells of mice and rats. It may be that heparin or the particular heparin fraction present in the mast cells of these rodents plays an important role.

Of course, storage of 5-HT in mast cells is strictly connected with the integrity of these cells: physiological or pathological factors or pharmacological agents which cause degranulation or disruption of mast cells inevitably liberate 5-HT, together with histamine and heparin. However, 5-HT release is not necessarily connected with gross morphological damage of mast cells.

While nothing is known about the eventual 'physiological' release of mast cell 5-HT, it has been ascertained that the amine can be set free from mast cells in the following ways:

- a) by the classic histamine releasers 48/80 and propamidine, added to suspensions of mast cells obtained from the peritoneal cavity, or injected intra-arterially into the perfused rat hindquarter [111, 112, 113, 29b], or subcutaneously into the dorsal skin of the rat paw [401, 907], or by the intraperitoneal route [461];
- b) by toluidine blue, d-tubocurarine, 1,10-diaminodecane, stilbamidine or pentamidine added to mast cell suspensions [461];
- c) by morphine given intra-arterially in perfusion experiments [114, 901, 907];
- d) by reserpine given systematically [113, 901, 907];
- e) by repeated parenteral administration of polymixin B [196];
- f) by dextran, egg-white, ovomucoid, testis extracts as well as alkaline extracts from several rat tissues injected into the dorsal skin of the paws, added to suspension or perfusion liquids, or given systematically [539, 905, 1010, 29b, 483b];
- g) finally, in the anaphylactic reaction produced, for example, by addition of antigen to isolated peritoneal cells taken from sensitized animals [461].

4.4 Uptake and release of 5-HT in the CNS

There is no evidence whatever demonstrating that the 5-HT synthesized within the CNS arrives, after having crossed the brain-blood barrier, into the general circulation.

The only thing we do know is that 5-HTP is taken up by brain tissue through an active transport mechanism [1060].

5. Biosynthesis and Metabolism of Indolealkylamines

It may be accepted with reasonable certainty that L-tryptophan is the remote precursor of all indole aminoacids and indolealkylamines bearing a three-carbon or two-carbon atom side chain at position 3 of the indole nucleus.

5.1 Conversion of L-tryptophan into tryptamine. Tryptamine derivatives

The decarboxylation of tryptophan to tryptamine was first described by WERLE [1308], and recently confirmed by HESS *et al.* [574] and WEISSBACH *et al.* [1288]. Both mammalian kidney and liver and human foecal bacteria were found to be capable of carrying out the reaction. On the other hand it has been shown that urine and tissues of mammals contain detectable amounts of tryptamine [991] and that there is a significant or even formidable increase in the tryptamine content of these biological materials following administration of L-tryptophan or iproniazid plus L-tryptophan [574]. This demonstrates that tryptophan decarboxylation does occur to an appreciable extent under normal conditions and that there is probably a high turnover of tryptamine present in the body [574].

It is so far difficult to decide whether *L-tryptophan decarboxylase* is identical or not with 5-HTP decarboxylase or DOPA decarboxylase.

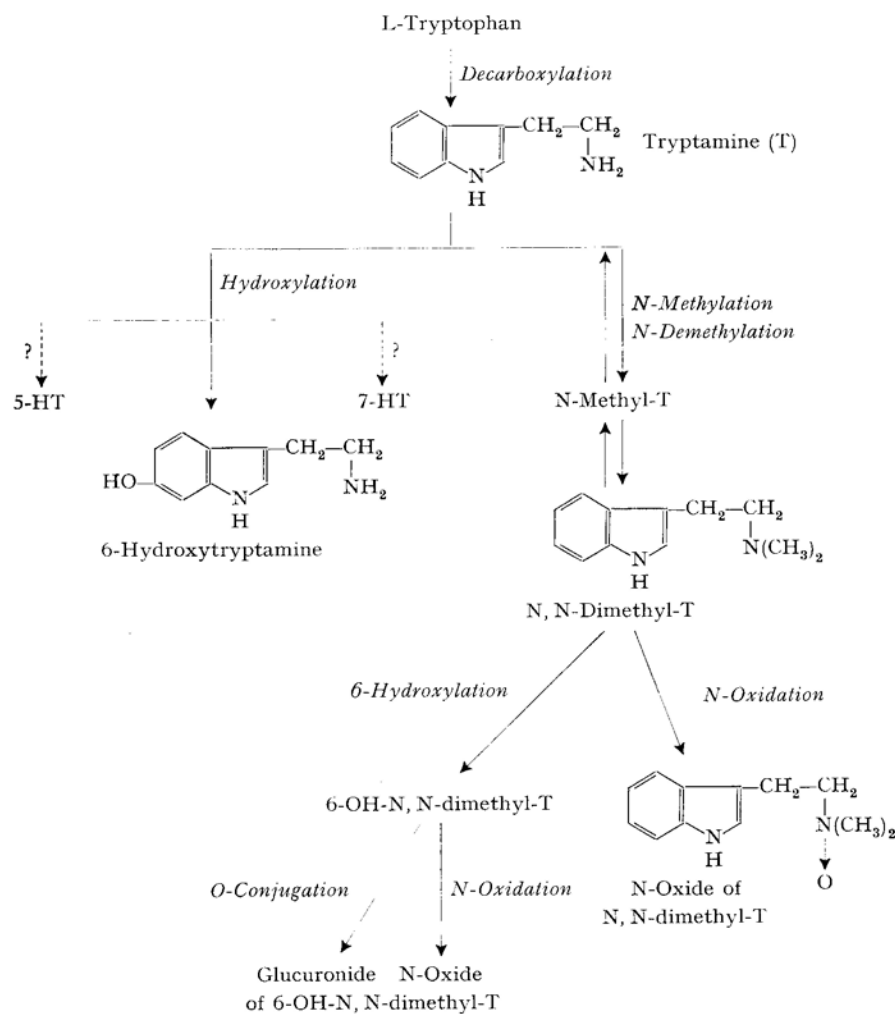
Exogenous and/or endogenous tryptamine may be hydroxylated in the indole nucleus as well as N-methylated or N-oxidated. This may be either *in vitro*, by enzyme preparations, *in vivo*, or both *in vitro* and *in vivo*. All the changes hitherto described as possibly occurring in nature are shown in Fig. 1.

The hydroxylation of tryptamine at the 5- and 7- position has been claimed to occur enzymatically in liver extracts by ICHIGARA *et al.* [608], but JEPSON *et al.* [619] were unable to duplicate the results of the Japanese investigators. They found that hydroxylation of tryptamine by washed rabbit liver microsomes gave 6-HT as the only product of consequence.

This has been confirmed *in vivo* by SZARA *et al.* [1185, 1186] who observed that in rats hydroxylation of both N,N-dimethyltryptamine and N,N-diethyltryptamine occurs only in the 6-position, and that this hydroxylation, followed by conjugation to glucuronic acid, represents the major pathway for the metabolism of the above amines as well as of α -methyltryptamine in rats.

In addition to 6-hydroxy- α -methyltryptamine and to the corresponding O-glucuronide, urine of rats injected with α -methyltryptamine contains 6-hydroxyindolylacetone and 6-hydroxyindolylacetone glucuronide [1186b]. The

Fig. 1
Derivatives of tryptamine



hydroxylating enzyme belongs to the family of microsomal enzymes requiring TPNH and O_2 for reaction. It is inhibited by chlorpromazine, reserpine and higher alkylated tryptamine derivatives (e.g. N,N-dihexyltryptamine [1186c]).

N-methylation of tryptamine has so far never been described in the animal organism, but only in plant tissues. N-demethylation of N,N-dimethyltryptamine, however, was shown to be carried out by mammalian tissues also, both *in vitro* and *in vivo* [1185]. Since adrenaline also may be demethylated *in vitro* by rat liver mitochondria [1335] and *in vivo* in the cat organism [1250] it seems that N-demethylation of biogenic amines may be considered as a process of general biological significance.

N-oxidation of tertiary amines is carried out by plant tissues and by mammalian enzyme preparations [1185, 424]; so far it has not been observed *in vivo* in the mammalian organism.

UDENFRIEND *et al.* [1233] decidedly exclude the possibility that in the mammalian organism tryptamine may be hydroxylated in the 5-position to give 5-HT, which would represent an important alternative pathway in the biosynthesis of 5-HT. However, in the writer's opinion (see also WOOLLEY) [1323] it would be better to leave this question open until more evidence is available.

In this regard it should be kept in mind that increased urinary excretion of 5-HIAA has been observed in man both after oral administration of high doses of indole-acetic acid [1019] and after intramuscular injection of N,N-dimethyl-tryptamine [1184]. If the excess urinary 5-HIAA really originated from the administered compounds, it is evident that at least a portion of them must have been hydroxylated at the 5-position.

5.2 Conversion of L-tryptophan into L-5-HTP and L-4-HTP

All the available evidence strongly suggests that 5-HTP and 4-HTP must be considered the immediate precursors of the biogenic 5-hydroxy- and 4-hydroxy-indolealkylamines, respectively.

In regard to 4-HTP it should immediately be added that so far this amino-acid has never been detected in the living organism, nor has the primary amine originating from it. However it is to be expected that extensive investigation of the vegetable kingdom, especially of fungi, will fill these gaps, thus proving the possibility of biogenic hydroxylation of L-tryptophan even in the 4-position.

In the case of 5-HTP things are simpler and clearer. First of all, 5-HTP has been found in considerable amounts in the urine of some patients suffering from carcinoidosis [1038, 1140, 335] and in one of these patients dietary ^{14}C -tryptophan was shown to have been converted into labelled 5-HTP [1125].

Secondly an enzyme system catalyzing the hydroxylation of tryptophan to 5-HTP has been found not only in *Chromobacterium violaceum* [837] but quite recently also in mammalian tissue. In fact the particulate fractions of both guinea-pig and rat intestinal mucosal cells were capable of transforming L-tryptophan into 5-HTP in the presence of ascorbic acid and Cu^{++} [268b].

This is not all, for in addition to the above two pieces of direct evidence, there is a great deal of strong indirect proof that L-tryptophan can be converted into 5-HTP and then into 5-HT.

a) In carcinoidosis, where an enormous accumulation of enterochromaffin cells exists, as much as 60% (instead of the normal 1%) of dietary L-tryptophan is found in the urine in the form of 5-hydroxy-indoles [1125]. Moreover, following administration of ^3C -DL-tryptophan to three carcinoid patients, radio-active 5-HIAA made its appearance in the urine [1225].

b) Typical enterochromaffin cells differentiated *in vitro* in organotypic cultures of intestine of chick embryos explanted before enterochromaffin cells are known to differentiate during the incubation period [838c]. This demonstrates that

5-HT is elaborated by the enterochromaffin cells themselves from the L-tryptophan available in the cultural medium.

c) Rats and other animals fed on a low-tryptophan diet showed a decrease of 5-HT in their gastro-intestinal tract [1167, 344, 1347], and of 5-HIAA in their urine [1206]; conversely, rats fed on a diet rich in tryptophan or given pure L-tryptophan displayed an increase in tissue 5-HT and urinary 5-HIAA [1167, 102, 1183c]. Similar observations have been made in man. Both healthy subjects [674, 704] and carcinoid patients [624, 1140] excreted more 5-hydroxyindoles in the urine after intake of tryptophan or a high protein diet, and less 5-HIAA when kept on low protein, high fat diets [1140].

d) Phenylacetic acid and α -methyl DOPA, which are known to be 5-HTP-decarboxylase inhibitors, reduced urinary output of 5-HT and 5-HIAA in patients with carcinoid tumour [1039, 1130b], while largely increasing urinary excretion of 5-HTP (from 1–6 to 55–74 mg per day) [1130b]; and in mice and guinea-pigs they produced a fall in brain and intestinal 5-HT concentrations [1137].

e) Neoplastic mast cells from a mouse mastocytoma cultured *in vitro* were capable of maintaining their high level of 5-HT after many generations in culture [1083, 1084], at the expense, of course, of the L-tryptophan in the culture medium.

Not much is known about the enzyme systems which catalyse the hydroxylation of L-tryptophan. From a theoretical point of view it must be admitted that besides highly specific hydroxylating mechanisms, non-specific hydroxylating enzymes [292, 293] also may carry out the transformation of L-tryptophan into 5-HTP. Nevertheless the latter are believed to be of practical significance only when the former are lacking due to congenital or acquired defects [293].

The specific hydroxylating enzyme (*L-tryptophan 5-hydroxylase*) in all probability has an extremely limited distribution and only tissues which contain this enzyme are capable of carrying out a complete biosynthesis of 5-HT. The restricted distribution of tryptophan hydroxylase contrasts sharply with the nearly ubiquitous distribution of the so-called 5-HTP decarboxylase and makes it likely that hydroxylation and not decarboxylation is the rate-controlling step in the formation of 5-HT [294, 295, 1227].

So far, in mammals, only three types of tissues have been shown to be capable of carrying out the hydroxylation of tryptophan: the gastro-intestinal mucosa, the nervous tissue and, only in rats and mice, the mast cells. However, it is highly probable that other tissues also are capable of hydroxylating tryptophan: cutaneous glands of the amphibian skin, posterior salivary glands of octopods, hypobranchial body of Muricidae, venom apparatus of wasps and scorpions, etc. In short it may be said that wherever there are chromaffin cells belonging to the enterochromaffin cell system there is the possibility of a complete biosynthesis of 5-HT.

5.3 Decarboxylation of 5-HTP

5-Hydroxytryptophan is decarboxylated to 5-HT by an enzyme system having a very widespread distribution in nature: the so-called *5-HT decarboxylase*. Only the L-form of 5-HTP has been found to be acted upon by the decarboxylase [441].

5.31 Substrate specificity of 5-HTP decarboxylase

This is the first fundamental problem one has to face when investigating the decarboxylation of 5-HTP. Briefly, the question is this: is 5-HTP decarboxylated by a strictly specific enzyme, or is 5-HTP decarboxylase nothing but the previously-known DOPA decarboxylase?

Nearly all recent investigators agree that mammalian 5-HTP decarboxylase and DOPA decarboxylase are one and the same thing. In fact:

1) The distribution of DOPA- and 5-HT-decarboxylase in mammalian tissues is exactly the same. This is true even when considering the different areas of the central nervous system [1306, 103, 534, 684b].

2) Decarboxylation of DOPA is competitively inhibited by 5-HTP and vice versa. Mixtures of 5-HTP and DOPA are found to be decarboxylated at a rate intermediate between the decarboxylation rates of the separate substrates and the depression in the decarboxylation rate of the mixture is roughly proportional to the molar ratio of 5-HTP and DOPA [103, 996, 1338]. This suggests that DOPA and 5-HTP are competing with the same enzyme.

3) Even after 82-fold purification of DOPA decarboxylase from guinea-pig kidney the ratio of the activities of the preparation towards DOPA, 3:4-dihydroxyphenylserine and 5-HTP remains constant [1309].

Similar results have been obtained by ROSENGREN [996] with the purification of an enzyme preparation made from rabbit kidney cortex by means of precipitation and chromatography.

It has been observed that the rate of enzyme activity towards 5-HTP and DOPA differs from organ to organ. The reason seems to lie mainly in the different amounts of co-enzyme in the organ extracts, since the addition of pyridoxal phosphate eliminates the difference [1306].

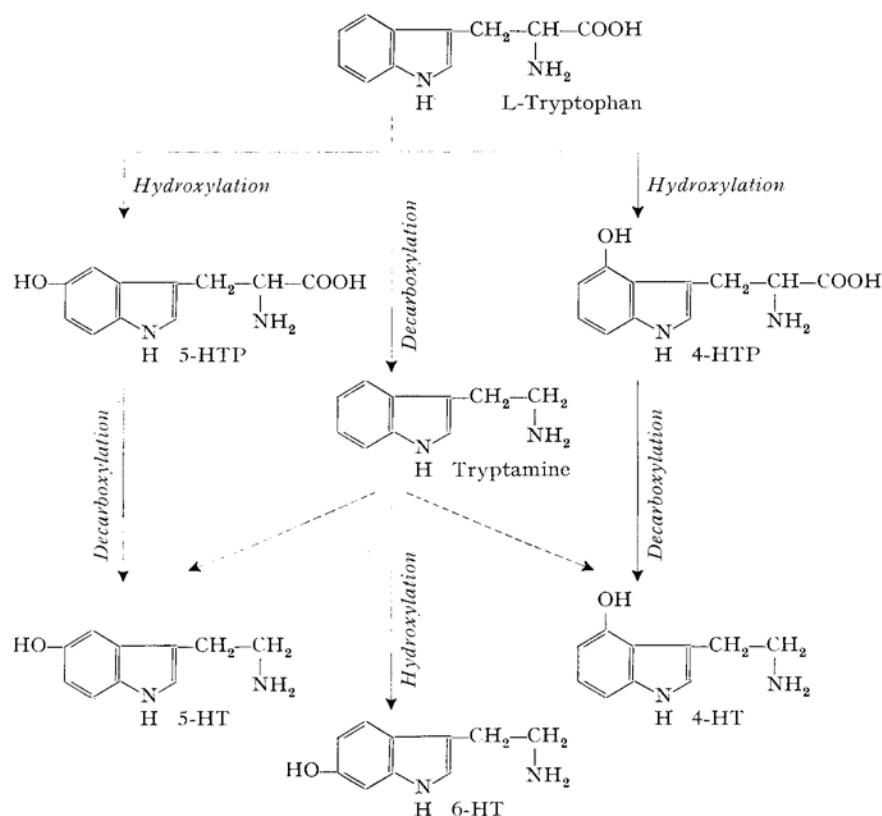
4) Inhibitors of DOPA decarboxylase also inhibit the decarboxylation of 5-HTP. This is true for α -methyl DOPA [1306, 1142], *m*-tyrosine, *o*-tyrosine, caffeic acid [996], carboxychalcones [1338, 1339], dopamine and other catecholamines, thiosemicarbazide [1338], iproniazid [894] and other substances.

5) Finally, 4-HTP is another excellent substrate for 5-HTP decarboxylase. This has been demonstrated both *in vitro* and *in vivo* [381] and this observation alone is sufficient to prove that in no case is 5-HTP decarboxylase an enzyme specific for 5-HTP.

Quite recently UDENFRIEND *et al.* [1234], on the basis of experimental evidence very similar to that reported above, not only accepted the identity of DOPA decarboxylase with 5-HTP decarboxylase, but, going much further, have suggested the existence of a single aromatic L-aminoacid decarboxylase. In the writer's opinion this unification of all the decarboxylating enzymes active

on aromatic aminoacids seems somewhat premature. It is not easy, for example, to accept *sic et simpliciter* that histidine is decarboxylated by the same enzyme which decarboxylates DOPA and 5-HTP at rates 1000 to 3000 times higher. Similarly there is no justification for the creation of new, separate decarboxylases: the latest example is that of DAVIS and AWAPARA [302] who suggest that DOPA and *o*-tyrosine are decarboxylated by different enzymes.

Fig. 2
Biosynthesis of naturally occurring primary hydroxyindolealkylamines



Among the numerous tryptophans hitherto tested, only 4-HTP and 5-HTP have been found to be acted upon directly by the decarboxylase. 5-Hydroxy-N-acetyl-TP and 5-acetoxy-N-acetyl-TP have been decarboxylated only after their hydrolytic conversion to 5-HTP. No acetylated tryptamines could be detected in the urine of rats given the two aminoacids [385].

The following tryptophans have not been decarboxylated *in vitro*, at least not at rates measurable in conventional Warburg apparatus, nor have they given origin in the rat organism to detectable amounts of the corresponding

amines; 7-HTP, 2-HTP, iso-5-HTP, 2,5-dihydroxy-TP [258, 441], 6-HTP, 6-benzyloxy-TP, 4-methyl-TP, 4-benzyloxy-TP, 5-methyl-TP, 5-methoxy-TP, 5-benzyloxy-TP, 5-hydroxy-N,N-dimethyl-TP, 5-hydroxy- β -methyl-TP, 5-hydroxy-6-methoxy-TP, 5-hydroxy-6-methyl-TP, 5-hydroxy-2-methyl-TP, 5-nitro-TP, 5-amino-TP, 5-benzyloxy-6-methoxy-TP [384].

The statement that in all probability mammalian DOPA decarboxylase and 5-HTP decarboxylase are identical does not imply that all enzymes capable of decarboxylating DOPA are active on hydroxytryptophans, too. ERSPAMER *et al.* [384] observed that a bacterial tyrosine-decarboxylase possessing a powerful action on DOPA was completely inactive on both 5-HTP and 4-HTP.

On the basis of all the above considerations, the name 5-HTP decarboxylase should be dropped as useless and confusing. It will be used in this paper only for the reason that the name DOPA decarboxylase raises similar objections and because the denomination aromatic L-aminoacid decarboxylase is still premature.

5.32 Distribution of 5-HTP-decarboxylase

Practically all parenchymatous tissues of mammals—gastro-intestinal tract, liver, lung, kidney, testicles, brain, sympathetic ganglia, etc.—possess a more or less intense 5-HTP-decarboxylase activity. This has been shown by studies *in vitro* and has further been proved by the accumulation of 5-HT in tissues of the intact animal after administration of 5-HTP.

Since 5-HTP decarboxylase is by no means a specific enzyme, it is not surprising that there is no correlation between its distribution and that of 5-HT [449, 140, 479]. The fact that several tissues lacking 5-HT (sympathetic ganglia and post-ganglionic sympathetic nerves, adrenal medulla, kidney, liver, testicles etc.) may still possess very high concentrations of 5-HTP-decarboxylase demonstrates that in these tissues the main function of the enzyme is to act on substrates other than 5-HTP. It is therefore nonsense to argue that a tissue showing 5-HTP decarboxylase activity must necessarily contain 5-HT.

According to HUANG *et al.* [602] 5-HTP decarboxylase activity is lacking in the rat kidney before and shortly after birth, and then gradually increases to adult levels over a period of several months.

5.33 Characteristics of 5-HT-decarboxylase

5-HTP decarboxylase is a rapidly-functioning enzyme which occurs exclusively in the non-particulate fraction of ultracentrifuged tissue homogenates [479, 536]. It requires pyridoxal-5-phosphate for its activity as a co-enzyme. This has been clearly demonstrated both *in vitro* and *in vivo*. In fact, kidneys from pyridoxine-deficient rats show lower 5-HTP-decarboxylase activity [198, 196] and, conversely, the addition of pyridoxine to the diet increases the concentration of 5-HTP-decarboxylase in the rat kidney [195].

BUZARD and NYTCH [197] suggest that the 5-HTP decarboxylase functions through an intermediate represented by a Schiff base formed by the combination of 5-HTP and pyridoxal phosphate in a fashion similar to that proposed

for other pyridoxal phosphate-depending decarboxylases. Thiol groups are not required for activity [195].

The pH optimum for enzyme activity has been found to vary between 7.5 and 8.1 [258, 694, 962, 536] or 7.0 and 7.5 [536].

5.34 Increase in blood and tissue indolealkylamines following administration of their precursor aminoacids

When ^{14}C -labelled 5-HTP is administered to rabbits, all the body depots of 5-HT become highly radioactive. When larger amounts (60–150 mg/kg) of non-isotopic 5-HT are administered, considerable amounts of 5-HT appear not only in the usual depots but in other tissues as well. In rats, for example, 5-HT levels rise from 0.1 to 6 $\mu\text{g/g}$ in the liver, from 0.1 to 3.1 $\mu\text{g/g}$ in the heart, from 0.2 to 2.5 $\mu\text{g/g}$ in the uterus and from 0.1 to 19 $\mu\text{g/g}$ in the kidney; in the dog the 5-HT concentration increases from 0.45 to 1.9 $\mu\text{g/ml}$ in the blood, from 0 to 1.5 $\mu\text{g/ml}$ in plasma, and from 0.1 to 6.2 $\mu\text{g/g}$ in the liver. Changes in the 5-HT content are less important in the gastro-intestinal tract [304, 1227].

The above results have been fully confirmed and extended by ERSPAMER *et al.* [386, 386b, 385, 381], who found that 60 minutes after the intraperitoneal administration to rats of 100 mg/kg of 5-HTP, 5-hydroxy-N-acetyl-TP, or 5-acetoxy-N-acetyl-TP, the 5-HT content increases in serum from 0.7 (control) to 1.68 (5-HTP), 1.28 (5-OH-N-acetyl-TP) and 0.93 $\mu\text{g/ml}$ (5-acetoxy-N-acetyl-TP); in kidney from 0.15 to 12, 12 and 23 $\mu\text{g/g}$, respectively, and in liver from 0.4 to 1.2, 0.83 and 0.78 $\mu\text{g/g}$, respectively. Maximum 5-HT increase is observed after one hour, then the amine concentration gradually declines and normal levels are generally attained within 16–24 hours. Only in the serum do 5-HT levels remain above normal for more than 48 hours.

One hour after the intraperitoneal injection of 100 mg/kg 4-HTP, the 4-HT levels in rat tissues were found to be as follows; intestinal tract 3–6 μg , liver 7–10 μg and kidney 40–60 $\mu\text{g/g}$. As expected, no 4-HT was found in the serum, probably owing to the incapacity of platelets to absorb the amine.

It is of considerable interest that 5-HTP administration to rabbits produces the appearance of 5-HT even in the adrenals (1.4 μg per pair of glands); more precisely, in the same granules which store catecholamines [104].

L-tryptophan alone (in doses up to 800 mg/kg) causes only a small increase in the tryptamine content of extracerebral tissues of the guinea-pig [574].

5.35 5-HTP decarboxylase inhibitors

Owing to its extreme importance, the study of 5-HTP decarboxylase inhibitors has attracted considerable attention and some noteworthy results have already been achieved.

The groups of inhibitors which so far have been most thoroughly examined, in consideration of their potency or their possible physiological significance are the following:

DOPA, 5-HTP and catecholamines. BERTER and ROSENGREN [103] found that repeated injections to rabbits of DOPA (600 mg/kg in total) lower the brain 5-HT level to 50–60% of the normal for two hours. Other investigators [1137, 1338] have observed that decarboxylation of 5-HTP may be inhibited *in vitro* not only by DOPA, but also by dopamine, adrenaline and noradrenaline and that, conversely, decarboxylation of DOPA is inhibited by 5-HTP but not by 5-HT.

According to BUZARD and NYTCH [197], noradrenaline is a non-specific formally non-competitive inhibitor of the dopadecarboxylase, acting through removal of the codecarboxylase present in the system.

These observations are obviously of conspicuous theoretical interest. In fact, the mutual inhibition of 5-HTP and DOPA decarboxylation by mixtures of these two substrates, and the inhibition of 5-HTP decarboxylation by the catecholamines, if operative *in vivo*, might have considerable physiological significance, producing a mechanism whereby a ratio of 5-HT and catecholamines is regulated, especially in the brain, by the available 5-HTP and DOPA.

α -methylDOPA. Up to the present this seems to be the most interesting decarboxylase inhibitor, active both *in vitro* and *in vivo*. *In vitro* the doses of α -methylDOPA producing 50% and 100% inhibition of the brain 5-HTP decarboxylase of mice were found to be 0.001 and 0.1 μ mole/ml, respectively, when no pyridoxal phosphate was present, and 0.1 and 10 μ moles/ml, respectively, when excess codecarboxylase was added. In all probability the inhibitor acts by co-enzyme inactivation.

In intact mice, guinea-pigs and dogs, single or repeated parenteral doses of α -methylDOPA (100–500 mg/kg) greatly inhibit cerebral and extracerebral dopadecarboxylase, producing a fall in the concentration of 5-HT, noradrenaline and dopamine in brain, heart and gastrointestinal tract [1137, 504b, 953b, 574b].

Similarly, α -methylDOPA prevents the central and peripheral effects produced by 5-HTP administration such as bronchospasm in guinea-pigs, central excitation in mice [1306], blood pressure increase in rats, guinea-pigs and spinal cats [322], as well as the cardiac stimulation produced in dogs by intravenous administration of DOPA or *m*-tyrosine [504b].

Whereas 5-HT and dopamine concentration returns to normal levels within 10 to 24 hours following the administration of α -methyl DOPA or α -methyl-*m*-tyrosine, depletion of noradrenaline persists for days [574b].

The above observations on experimental animals were extended to man by OATES *et al.* [868, 868b, 1130b], who found that 2 g of α -methylDOPA orally produces in hypertensive patients, together with a constant reduction of the average blood pressure, a 50 to 80% inhibition of the decarboxylation of administered 5-HTP, DOPA, *p*-tyrosine and tryptophan, and that 5 to 6 g α -methylDOPA decreases urinary 5-HIAA in carcinoid patients by 70% and increases 5-HTP excretion up to 60 mg per day.

Similar to α -methylDOPA in its capability of blocking decarboxylation of 5-HTP is α -methyl-*m*-tyrosine [504b, 953b], as well as 5-hydroxy- α -methyl-

tryptophan and, to a lesser degree, α -methyltryptophan, 5-hydroxy-1, α -dimethyltryptophan and 5-hydroxy-2, α -dimethyltryptophan. Surprisingly enough 5-hydroxy- α -methyltryptophan does not appear to be absorbed orally, at least in rats [561].

Other minor inhibitors belonging to this group are α -methyl-5-HT and α -methyltryptamine [515], as well as some additional tryptamines and tryptophans [441].

Phenylalanine metabolites. Phenylalanine metabolites have been studied on the basis of the observation that phenylketonuric patients show decreased production of 5-HT [306]. All three metabolites tested (phenylacetic, phenylpyruvic and phenyllactic acids) proved to be moderate inhibitors of 5-HTP decarboxylation *in vitro*, confirming the previous results of FELLMAN [404] on the decarboxylation of DOPA.

Moreover, in a patient with a 5-HTP-secreting carcinoid, SANDLER and CLOSE [1039] found that administration of 5 g phenylacetic acid by mouth appeared to decrease urinary output of 5-HT (up to 75%) and 5-HIAA (up to 25–30%).

However, TASHIAN [1190] obtained more ambiguous results in healthy human subjects, which cast some doubt on the actual efficacy of the phenylalanine metabolites as inhibitors of 5-HTP decarboxylase. He found that following oral administration of 20 mg/kg phenylacetic or phenylpyruvic acid or of 2 mg/kg *o*-hydroxyphenylacetic acid there was not a decrease but an increase in the urinary excretion of both indoleacetic acid and 5-HIAA.

Isoniazid. Starting from the fact that isoniazid forms a Schiff base complex with pyridoxal phosphate, thus depriving a decarboxylase or transaminase of the required co-factor, LUDWIG [752] tried the compound with success in carcinoid patients. After administration of 400 mg of the drug daily there was not only general clinical improvement, but also a 30–40% decrease of the 5-HT concentration in blood and a similar decrease of 5-HIAA in urine.

Carboxychalcones. 3,4,4'-trihydroxy-3'-carboxychalcone, 3,4'-dihydroxy-3'-carboxychalcone and similar compounds are potent competitive inhibitors of 5-HTP decarboxylase *in vitro* [1337, 1338]. In fact, virtually complete inhibition of the enzyme has been obtained by trihydroxychalcone at concentrations of 4×10^{-4} M [1339].

Analogues of hydroxycinnamic acids. These compounds have been thoroughly studied by CLARK *et al.* [257, 551], who found that 5-(3,4-dihydroxy-cinnamoyl) salicylic acid, 5-(3,4-diacetoxycinnamoyl)acetylsalicylic acid and the corresponding 3-hydroxy and 3-acetoxy acids are particularly active DOPA decarboxylase inhibitors *in vitro*, whereas caffeic acid, chlorogenic acid and 3-hydroxycinnamic acid are good inhibitors *in vivo* also.

To block the pressor response to 1–10 mg/kg L-DOPA, from 35 to 100 mg/kg of the best inhibitors is usually needed and, owing to rapid detoxication of the compounds, inhibition ceases within 30 minutes.

Inhibition seems to be 'competitive' with these compounds, too.

In addition to DOPA, caffeic acid and cynarine strongly inhibit the decarboxylation of 5-HTP and 4-HTP [384].

Additional inhibitors. Owing to the identity of DOPA decarboxylase with 5-HTP decarboxylase, all agents which were found to block decarboxylation of DOPA are, in all probability, also capable of inhibiting the decarboxylation of 5-HTP and 4-HTP. For a complete list and a discussion of these agents we refer the reader to the review article by CLARK [257].

Here we wish only to call attention to a few additional groups of compounds which have already been studied as 5-HTP decarboxylase inhibitors. They are only of limited theoretical interest, and their potency is rather low: this is true for chelating agents and carbonyl reagents [74], as well as for iproniazid and β -phenylisopropylhydrazine [894, 341], generally known as powerful monoamineoxidase inhibitors.

It has been pointed out by several investigators that inhibitors of 5-HTP decarboxylase, and especially α -methylDOPA, may provide a method of depleting tissues of 5-HT and other amines without the use of releasing agents such as *Rauwolfia* alkaloids, and may help in distinguishing some of the functions of 5-HT from those possessed by catecholamines. Also, they may offer a basic approach to investigation of blood pressure regulation and, finally, may provide a possible mode of treatment for human subjects suffering from diseases characterized by relative or absolute excess of amines.

All the above may certainly be true, provided that one keeps firmly in mind that 5-HTP decarboxylase is by no means a specific enzyme and, consequently, that inhibitors of the enzyme block the formation of a number of biogenic amines. It is already certain that not only 5-HTP and DOPA, but also *m*-tyrosine, *o*-tyrosine, the dihydroxyphenylserines and 4-HTP are substrates for 5-HTP decarboxylase and, should the hypothesis of UDENFRIEND *et al.* [1234] on the existence of a single aromatic L-aminoacid decarboxylase be proved to be correct, this list ought to be considerably lengthened, with the inclusion of aminoacids of such importance as *p*-tyrosine, phenylalanine, tryptophan and histidine.

There is no need to emphasize the complexity of the biochemical phenomena which may result from blocking the decarboxylation of so many amino-acids and, similarly, it appears quite obvious that the result of a decarboxylase block can vary greatly in different tissues depending on the spectrum of substrate aminoacids and on the intensity of enzymic activity.

In other words, here again, as in the case of MAO inhibitors and amine releasers, it is probable that 5-HTP decarboxylase inhibitors may prove of enormous importance in solving biochemical problems, but much less so in solving problems connected with the biological significance of single biogenic amines.

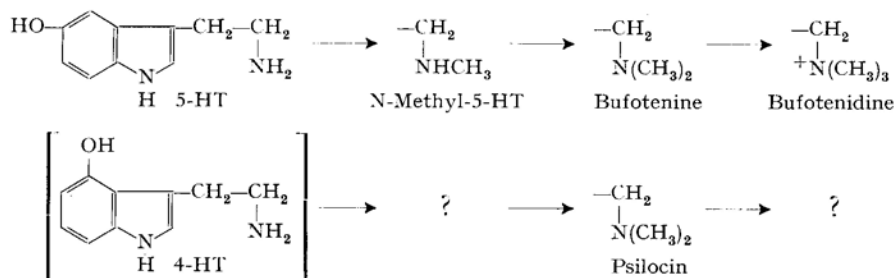
Attention has recently been called to an alternative metabolic pathway for 5-HTP, involving first a conversion of the aminoacid to 5-hydroxyindole-

pyruvic acid by a transaminase, and then a conversion to 5-hydroxyindolelactic acid, catalysed by a lactic dehydrogenase. The biological importance of this pathway is unknown [1161 c].

5.4 Metabolic pathways leading to 5-HT and 4-HT derivatives still bearing the amino group in the side chain

Metabolites of this type may originate through reactions involving the side chain or the indole nucleus, with particular regard to the free phenolic hydroxy group, or both. Only a few of the enzyme systems catalysing these reactions have been studied with sufficient accuracy.

N-methylation. This reaction, catalysed by a *N*-methyltransferase, causes the transfer of one, two or three methyl groups to the amino group, as shown below.



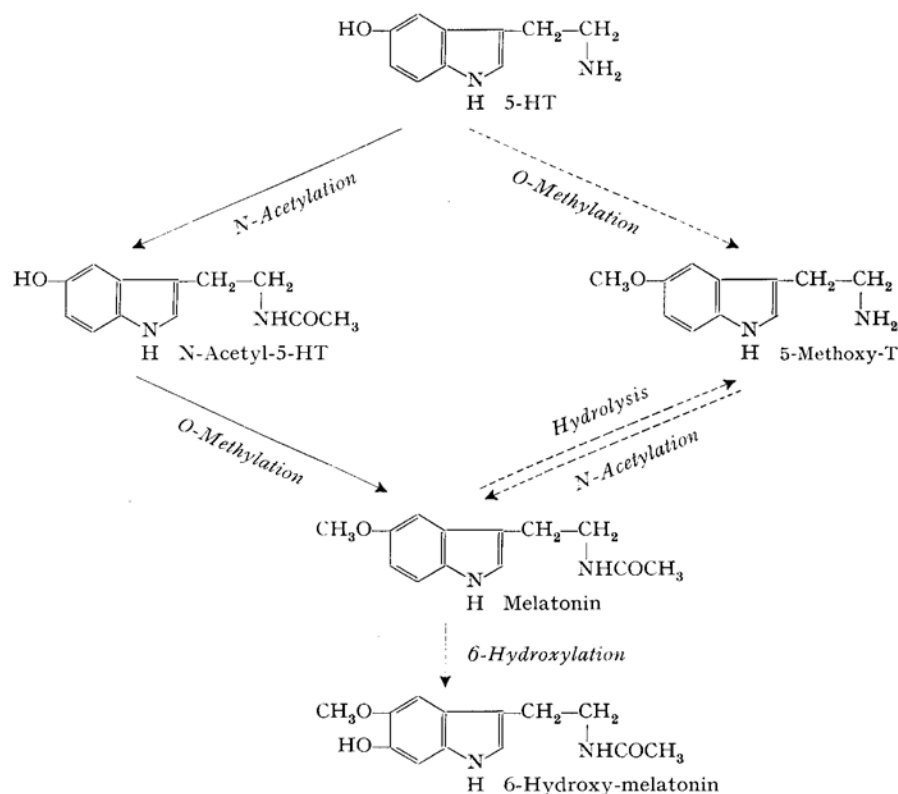
N-methylation processes have been found to occur in lower vertebrates (amphibians) and in plants (superior plants and fungi), but not in mammals. Previous reports on the presence of *N*-methylated derivatives of 5-HT in mammalian urine [190] have not been confirmed.

N-acetylation. According to McISAAC and PAGE [810], 5–25% of the administered ^{14}C -5-HT could be recovered from rat urine as *N*-acetyl-5-HT, 2% from rabbit urine. In addition, *N*-acetyl-5-HT has been shown to be a normal metabolite in the urine of carcinoid patients [809].

Although the recoveries reported by McISAAC and PAGE seem exceedingly high (KEGLEVIC *et al.* [640] were unable to find any *N*-acetyl-5-HT in the urine of rats given radioactive 5-HT, WEISSBACH *et al.* [1291 d] found only a small amount of the metabolite in mice given 100 mg/kg 5-HT, and ERSPAMER and BERTACCINI [386] had uncertain results in rats given enormous amounts of 5-HTP), it appears highly probable that *N*-acetylation of 5-HT does actually occur in the mammalian organism. In fact, *N*-acetyl-5-HT is considered by AXELROD and WEISSBACH [43] a better candidate than 5-methoxytryptamine as a precursor of melatonin. In favour of this assumption it may be stated that *N*-acetyl-5-HT is a much better substrate for hydroxyindole-*O*-methyltransferase than 5-HT; against it is the fact that 5-methoxy-indoleacetic acid, the deamination product of 5-methoxytryptamine, is, in contrast to *N*-acetyl-5-HT, a normal constituent of the bovine pineal gland [723].

Quite recently WEISSBACH *et al.* [1291] succeeded in demonstrating that rat liver and bovine pineal gland contain an enzyme which can acetylate 5-HT in the presence of an acetyl-coenzyme-A-generating system.

Fig. 3
Biosynthesis of melatonin



Dehydrogenation. Dehydrogenation of indolealkylamines has so far been demonstrated only in the skin of some amphibians (several *Bufo* species), and the only example is bufotenine, which is converted into dehydrobufotenine as follows:



O-methylation. The discovery of melatonin by LERNER *et al.* [718], and its identification as 5-methoxy-N-acetyltryptamine has proved definitely that 5-HT also may be O-methylated [721], exactly like catecholamines [41].

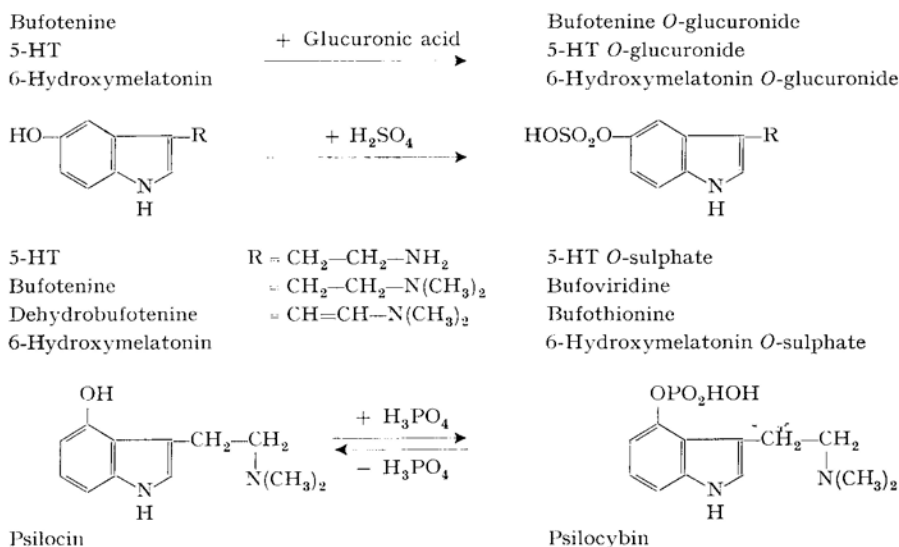
AXELROD and WEISSBACH [43] have now succeeded in demonstrating the presence of an enzyme, *hydroxyindole-O-methyltransferase*, in the cow and monkey pineal glands, which forms melatonin by transferring a methyl group from S-adenosylmethionine to the hydroxy group of N-acetyl-5-HT. The enzyme could not be detected in a variety of other mammalian tissues and appears to be highly localized in the pineal region.

The substrate specificity for hydroxyindole-O-methyltransferase can be expressed by taking as 100 that of N-acetyl-5-HT, whereupon bufotenine 14, 5-HIAA 12, N-methyl-5-HT 9, 5-HT 7, N-acetyl-4-HT 9 and 4-HT 0 would then be the relative values [43c].

Whereas O-methylation seems to be a major pathway for the metabolism of catecholamines [45, 42] this is not, generally speaking, the case with hydroxyindolealkylamines, although there may be important exceptions. LERNER *et al.* [723] isolated 40 μg melatonin from 100 g lyophilized bovine pineal glands (approximately 0.04–0.06 $\mu\text{g/g}$ fresh tissue). Since bovine pineal glands contain 0.4 $\mu\text{g/g}$ 5-HT [480] one can see that in this particular localization the O-methylated derivative accounts for 10 to 15% of the total hydroxyindolealkylamines. The same may be true of peripheral nerves in which no 5-HT but very minute amounts of melatonin are claimed to be present [722].

BACQ *et al.* [45] first demonstrated that catechol is a powerful inhibitor of catechol-O-methyl transferase. This has been confirmed by AXELROD *et al.* [40] and by UDENFRIEND *et al.* [1231b] who found that pyrogallol, glycoxyamine and quercetin are also similarly active.

It is evident that agents which block catechol-O-methyltransferase may also be of interest in the field of 5-hydroxyindolealkylamines as inhibitors of melatonin formation.



O-conjugation with organic and inorganic acids. Up to now, only glucuronic, sulphuric and orthophosphoric acids have been found to conjugate to hydroxyindolealkylamines in the living organism.

At the moment psilocybin is the only example of *O*-conjugation with phosphoric acid. Using purified intestinal phosphatase, psilocybin may be transformed to psilocin and inorganic phosphate [600c]. *O*-conjugation of hydroxyindolealkylamines with sulphuric acid is particularly intense in the skin of some amphibians. However, varying amounts of 5-HT *O*-sulphate could be found in mammalian urine also, following injection of high doses of 5-HT or 5-HTP [810, 250]. The same sulphate has been formed by incubation of 5-HT with rat liver homogenates [250]. A protein fraction prepared from these homogenates by differential centrifuging and salt precipitation has been shown to possess sulphate synthesizing activity. Essential requirements are adenosine triphosphate, Mg^{2+} and SO_4^{2-} ions.

Experiments with rats pretreated with iproniazid suggest that sulphate formation may be an alternative pathway for the metabolism of 5-HT when the normal route involving amine oxidase is inhibited or saturated. In fact, percentage excretion of 5-HT as 5-HT-*O*-sulphate increased from 7–9 to 20% following iproniazid premedication.

In the rat the availability of SO_4^{2-} ions proved to be a limiting factor for 5-hydroxytryptamine *O*-sulphate production [250].

At partial variance with the above positive results of CHADWICK and WILKINSON, ERSPAMER and co-workers [386, 386b] were unable to find in urine of rats given 5-HTP and 4-HTP by different routes detectable amounts of the *O*-sulphates of the corresponding amines.

Similarly to 5-HT, a portion of administered melatonin may be recovered in rat and man [722b] urine as the sulphate conjugate of *N*-acetyl-5-methoxy-6-hydroxytryptamine [675].

WEISSBACH *et al.* [1287, 1291d] affirmed that approximately 25–30% of the administered 5-HT is recovered in mouse urine as the 5-HT-*O*-glucuronide; however, McISAAC and PAGE [810] found that in other species the yield in the glucuronide conjugate of 5-HT is extremely variable, ranging from 0 to 30% in rat urine, and from 0 to 12% in rabbit urine.

Following administration to rats of 5-HT, 4-HT, bufotenine, 5-HTP and 4-HTP, the corresponding glucuronides could be detected not only in the urine but also in blood serum, liver and kidney [381, 386, 386b, 489b].

Finally, *O*-conjugation with glucuronic acid seems to be quite common for the 6-hydroxytryptamines [1186]. Again, in the field of biogenic amines, one of the melatonin metabolites found in rat urine was the *O*-glucuronide of *N*-acetyl-5-methoxy-6-hydroxytryptamine [675].

It may be concluded that, similarly to and perhaps more than *O*-conjugation with sulphuric acid, conjugation with glucuronic acid may represent an important metabolic pathway for hydroxyindolealkylamines in the mammalian organism.

Introduction of a second hydroxy group into the indole nucleus. There is no definite evidence that 5-HT or related hydroxyindolealkylamines may be further hydroxylated in the nucleus, thus giving origin to dihydroxyindolealkylamines. This possibility, however, has been repeatedly put forward [226], and 6-hydroxylation has been regarded as being more likely to occur [296, 130]. In confirmation of this assumption, not only tryptamines, as already mentioned, but also melatonin was found to be hydroxylated in the 6-position [675, 722b], and 6-hydroxymelatonin is now considered to be the main metabolite of this hormone.

Hydroxylation in the side chain. No evidence of a biologically occurring hydroxylation of indolealkylamines in the side chain, similar to that observed for catecholamines [727], is available.

On the usual smooth muscle preparations, used in the bioassay of 5-HT, the β -hydroxylated derivative of bufotenine proved to be more than 100 times less active than its parent substance [100].

The metabolic changes of 5-HT and 4-HT described above are generally considered, even at the present time, to be processes leading to more or less complete biological inactivation of the amines.

Some recent findings point the way to a radical re-consideration of these concepts. It is sufficient to stress that even O-conjugation with inorganic acids or O-methylation may lead to derivatives which, contrary to expectation, are as active as the parent amine or, in some respects, thousands of times more active. Psilocybin, for example, is exactly as active as psilocin, both in its peripheral and central effects [1280], and melatonin is 50,000 times as potent as 5-hydroxy-N-acetyltryptamine in causing lightening of the frog skin [215, 722b].

N-acetyl-5-HT, in its turn, is 200 times more active, on the same test object, than 5-HT, thus demonstrating that N-acetylation also, while destroying some biological actions of 5-HT, enormously potentiates other effects.

It therefore seems prudent not to confound the metabolism of an amine with its inactivation, and to keep in mind that, under different circumstances and in different sites, the same compound may be the active substance itself, its precursor or even its inactive derivative.

It should also be pointed out that the apparent pharmacological inactivity of a metabolite may be due only to its inability to reach the effector structures in sufficient concentration or to the fact that it may cause pharmacological effects other than those recorded by the methods employed.

5.5 Oxidative deamination

It is certain that one of the most important pathways for the degradation and inactivation of indolealkylamines in the organism is represented by oxidative deamination, carried out both by soluble and insoluble (mitochondrial) monoamine oxidase (MAO) systems.

For the sake of simplicity we refer the reader to BLASCHKO *et al.* [129] in regard to the classification of MAO, based on substrate and on inhibitory specificity. The oldest and best known MAO is the so-called 'insoluble' or 'mitochondrial' MAO, contained in the mitochondrial portion of a variety of tissue homogenates.

5.51 Distribution of MAO

'Mitochondrial' MAO is widely distributed in nearly all parenchymatous tissues of vertebrates and in a number of tissues of invertebrates. Particular attention has been paid to the distribution of the enzyme in mammalian brain and the conclusion is that MAO is relatively evenly distributed throughout the brain, being somewhat higher in the thalamic and hypothalamic regions and lower in the white matter [140, 139, 1293].

An insoluble MAO is perhaps also present in the cellular elements of the blood [1267], and a soluble MAO has been described in guinea-pig liver [1286], and in the plasma of several mammals [129].

MAO may be histochemically visualized in tissues by tetrazolium salts and other methods [30, 495, 1333], and tetrazolium reduction has been suggested as an additional measure of MAO activity *in vitro* [1285]. Using the tryptamine-tetrazolium method, SHIMIZU and MORIKAWA [1105] demonstrated that with the exception of *locus coeruleus* and *nucleus ambiguus*, which display a MAO reaction already in late foetal life, the remaining portions of the rat brain are practically negative for MAO activity until new-born life, then rapidly reaching adult activity within 3 weeks after birth.

There is no need to stress the value of studies on the embryologic development of enzymes interfering with biosynthesis and metabolism of biogenic amines considered as possible neurohumors.

Following chronic administration of high doses of 5-HT to rats, decreased MAO activity was found in the liver of treated animals [695].

Similarly, an inhibitory influence on MAO and other enzyme systems interfering with the metabolism of 5-HT may be seen in mice given sublethal doses of ethanol [998].

5.52 Substrate specificity of MAO

Mitochondrial MAO provokes the oxidative deamination of a great number of aliphatic and aromatic amines of biological interest, among which are found not only phenylalkylamines and indolealkylamines, but also imidazolealkylamines [1350, 1293b].

To confine our attention to the field of indolealkylamines it may be seen that all compounds bearing a 2 carbon-atom side chain with the amino group attached to the second carbon atom can be oxidized by MAO. Primary amines are better substrates than secondary amines and still better than tertiary amines. Quaternary ammonium bases are not inactivated by the enzyme.

5-methoxytryptamine and tryptamine as substrates for MAO are as good as, or better than 5-HT, whereas 4-HT, 6-HT and 7-HT are oxidized by guinea-

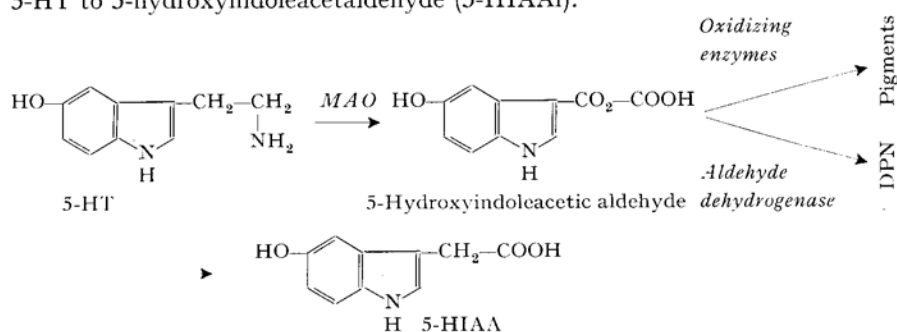
pig kidney or liver MAO at about 50–75% of the rate of 5-HT. Iso-tryptamine and iso-5-HT are poor substrates for MAO and β -hydroxybufotenine apparently is not attacked by the enzyme [383].

N,N-dimethyltryptamine-N-oxide is a weak substrate of MAO, the oxidation of the compound proceeding best when the oxygen tension is reduced [1142c].

It is obvious that a great number of amines can be used as substrates in *in vitro* studies on the MAO activity of tissue preparations. Among these substrates, kynuramine seems to offer the advantage that its disappearance from the reaction mixture, following its conversion to 4-hydroxyquinoline, may be quantitatively estimated by a simple and rapid spectrophotometric procedure [1289].

5.53 Properties and actions of MAO

Using the soluble MAO of guinea-pig liver as the enzyme preparation and 5-HT as substrate, WEISSBACH *et al.* [1286] succeeded in demonstrating that the fundamental action of MAO is to produce the oxidative deamination of 5-HT to 5-hydroxyindoleacetaldehyde (5-HIAA).



5-HIAAal may then be converted either to 5-HIAA by a diphosphopyridine nucleotide-linked aldehyde dehydrogenase or to pigments by oxidizing enzymes. The rate-limiting step in the formation of 5-HIAA from 5-HT seems thus to be the dehydrogenation of 5-HIAAal. When aldehyde dehydrogenase is abundant or active, as in the soluble MAO system, or if an aldehyde dehydrogenase preparation or the venom of *Crotalus atrox* [548] is added to the mitochondrial system, then there is much 5-HIAA formation, with inhibition of pigment formation; otherwise, as in the mitochondrial MAO system alone, a considerable part of 5-HIAAal is rapidly oxidized to pigments and the yield in 5-HIAA decreases.

Under the most favourable conditions WEISSBACH *et al.* [1286] obtained a quantitative conversion of 5-HT into 5-HIAA, with a stoichiometric reduction of DPN.

A screening method for the rapid detection of MAO inhibitors is based upon the inhibition of the appearance of the dark brown pigment which is regularly produced by the action of MAO on 5-HT [1332].

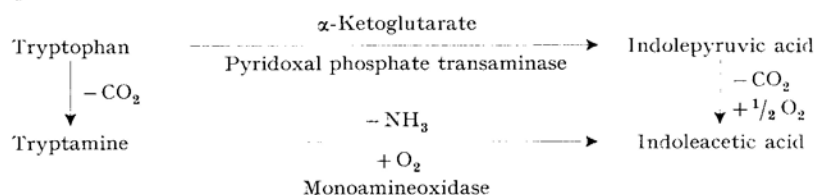
The only metabolite resulting from the oxidative deamination of the indolealkylamines which it has so far been possible to trace in the organism is the acid. The corresponding aldehyde could only be traced *in vitro*. Acids deriving from endogenous indolealkylamines are generally detectable only in urine; acids deriving from exogenous amines or precursor aminoacids administered in large doses may be found in blood and tissues also.

MAO is an enzyme acting promptly. In fact, SCHAYER *et al.* [1063] observed that out of intravenously injected radioactive tryptamine as much as 74% was metabolized in mice within 90 seconds. This percentage dropped to 31 after iproniazid pretreatment.

5.54 Urinary indole acids

The following acids have been found by ARMSTRONG *et al.* [32] in human urine: 5-HIAA, indoleacetic acid (IAA), indolelactic acid, indoleacetylglutamic acid, indole-3-carboxylic acid, indoleacrylic acid and indoleglycolic acid. It is very difficult to state the origin of these compounds. A dietary origin seems possible for practically all of them. This is true even for 5-HIAA and IAA which may increase considerably after ingesting bananas or vegetables containing high amounts of tryptamine or 5-HT [16, 733, 964, 229].

However, apart from this particular circumstance, it is probable that all urinary IAA and, still more, all 5-HIAA is of endogenous origin. The bulk of IAA probably arises from tryptamine by transamination, via the indolepyruvic acid, and only a minor part arises from tryptamine by oxidative deamination [1288].



The daily urinary excretion of IAA in man is generally in the range of 5–18 mg per day and may be as high as 200 mg daily in certain pathological conditions. As expected, the excretion of IAA may be increased markedly by tryptophan loading [1288], and still more by administration of tryptamine. IAA is present in urine either free or in the form of its glycine conjugate, indoleaceturic acid. Following subcutaneous injections of tryptamine, N-methyltryptamine and N,N-dimethyltryptamine the percentage recovery of the amines as IAA have been found to be 84.6, 35.5 and 17.4 respectively, and the ratio of conjugated: free acid ranged from 2.2 to 5.4 [365].

In contrast to IAA, it is generally believed that practically all urinary 5-HIAA is derived from the oxidative deamination of endogenous 5-HT and, consequently, that urinary 5-HIAA may truly reflect the rate of biosynthesis of 5-HT. The possibility that 5-HIAA arises from indoleacetic acid either

through specific or unspecific hydroxylation reactions [292, 1040] or directly from 5-HTP, bypassing 5-HT, through oxidation catalysed by L- and D-aminoacid oxidases [127, 1224], although repeatedly postulated, seems to be of only theoretical interest.

The daily urinary excretion of 5-HIAA in man and experimental animals is as follows: man 1–8 mg [365, 1145, 1124, 1125, 696, 916], cat 25–40 $\mu\text{g/kg}$ [386], dog 40–120 $\mu\text{g/kg}$ [368], rat 80–230 $\mu\text{g/kg}$ [365, 99, 409].

Urinary 5-HIAA excretion in man seems to be particularly low at birth (0.17 mg/kg/day), but values close to those seen in adulthood (0.35 mg/kg/day) are reached within a few months [1090, 1091].

Diurnal variations in the urinary excretion of 5-hydroxyindoles have been demonstrated in man by JOHNSON *et al.* [622]. They found that the lowest concentration of the acid in urine occurred between midnight and 3 a.m., and the peak in the 3-hour period on either side of noon.

The excretion rate of 5-HIAA originating from endogenous 5-HT is not significantly changed in man with varying urinary pH [831b].

In rats no significant change in 5-HIAA excretion is observed following 5-HT administration, but following 5-HTP administration «acid rats» excrete less 5-HIAA than «alkaline rats» [1040b].

It is evident that changes in the release of 5-HT from the body stores (e.g. administration of 5-HT releasers) or in the production of endogenous 5-HT (e.g. carcinoidosis) provoke corresponding changes in the urinary output of 5-HIAA; and it is similarly obvious that the 5-HIAA content of urine may rise conspicuously after administration of 5-HT or 5-HTP. From 22 to 47% of the 5-HT administered by the parenteral route [365, 368, 308] and 50–75% of the amine given by mouth [733, 118] could be recovered in man as 5-HIAA. Recoveries in dogs were 20–37% [365, 368, 1224], in cats 10–15% (ERSPAMER, unpublished), in rabbits 3–4% [859] to 83% [810] after administration of 5-HT by the parenteral route; in rats 28–67% when 5-HT was given parenterally [365, 378, 810, 409, 250, 9], but only 5.5% when given by mouth [365]; in mice 30% of the administered 5-HT appeared in urine as free 5-HIAA and 10% as 5-HIAA glucuronide [1291d].

Furthermore in rats the yield of 5-HIAA was twice as much (47–65%) following subcutaneous injection of 5-HT, as when following intravenous injection (23–35%). No such differences could be demonstrated in animals pretreated with iproniazid [8].

Following administration of 5-HTP, urinary recoveries as 5-HIAA, referred to the racemic aminoacid, were 4–18% in man [304, 662c] and 5–15% and 3–5% in the rat after intraperitoneal injection and oral administration, respectively [386].

5-HIAA may be found in urine in a free form, or conjugated with glycine [810], with glucuronic acid [386], or with sulphuric acid [289]. The ratio of free 5-HIAA: 5-hydroxyindoleacetic acid was found by MCISAAC and PAGE [810] to be approximately 1:6 in rat urine and only 0:06 in rabbit urine. However, at partial variance with these findings, CHADWICK and WILKINSON [250]

affirm that about 70% of the 5-HIAA appearing in the rat urine after 5-HT administration is excreted as ester sulphate.

5-HIAA arises from the oxidative deamination not only of 5-HT, but also of N-methyl-5-HT and bufotenine (recovery 10%).

Other acids found in the urine after the administration of their precursors were: 4-HIAA from 4-HT and 4-HTP, 6-HIAA from 6-HT, 7-HIAA from 7-HT and 5-methoxy-IAA from 5-methoxy-T [365, 386, 813].

Following administration of DL-4-HTP urinary recovery of the aminoacid as 4-HIAA was 6-20% in rats and 10-13% in man [381b, 386b].

According to DESPOPOULOS and WEISSBACH [324] the renal elimination of 5-HIAA is achieved, like that of PAS, both by glomerular filtration and active tubular secretion. In fact, the intravenous administration of 2 g probenecid to carcinoid patients is followed by a decrease in the rate of excretion of 5-HIAA and an increase in its concentration in plasma.

5.55 MAO inhibitors

The subject of MAO inhibitors has recently created enormous interest, not only from a theoretical point of view, but also from a practical, therapeutical one.

The topic has been reviewed in a thorough and excellent way by PLETSCHER *et al.* [948] in the second volume of this series. Thus, we refer the reader to this review for more detailed information. Here, and again later on when we deal with the actions of MAO inhibitors on the CNS, we shall restrict the discussion of this subject to some of the essential points.

The main MAO inhibitors: Every list of MAO inhibitors should be considered as provisional, since it is continuously changing and lengthening in consequence of the efforts of several groups of research workers. For the time being the most representative drugs of this group may be listed as follows:

Iproniazid. (N²-isopropylhydrazide of isonicotinic acid; Marsilid®).

This is the prototype of the slow-acting or irreversible MAO inhibitors. Other interesting members of this series are the N²-isopropylhydrazide of L-serine, and the N²-isopropylhydrazide of L-glutamic acid [1259, 941, 944].

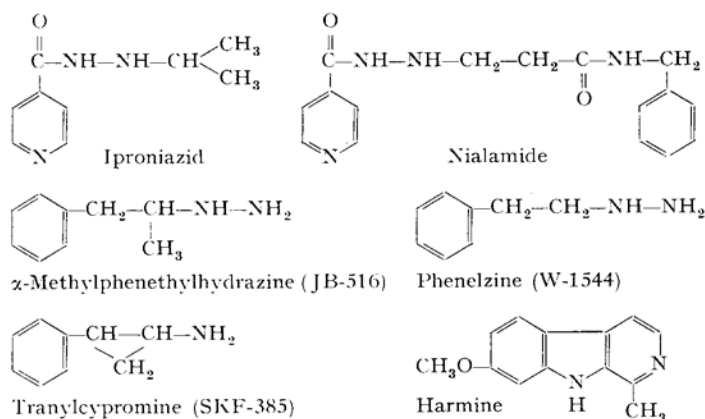
α-Methylphenethylhydrazine (Catron; JB 516; β-phenylisopropylhydrazine or PIH).

This is a rapid-acting, long-lasting inhibitor which is approximately equi-active to iproniazid as an *in vivo* inhibitor of liver MAO, but is 50 times more active than iproniazid on brain MAO. Subcutaneous PIH reduces the ability of brain MAO to metabolize 5-HT by approximately 65% within 15 minutes and maximal inhibition is obtained after 30-60 minutes. For iproniazid, on the other hand, maximal inhibition of brain MAO is seen only after 4 hours [502].

The properties of PIH are shared by other phenylhydrazines and benzylhydrazines: JB 835 (β-phenylbutylhydrazine), JB 821 (α-isonicotinyl-β-phenylisopropylhydrazine), Phenelzine (W1544, β-phenylethylhydrazine), Nialamide (N-isonicotinyl-N' [(β-N-benzylcarboxamido-ethyl)] hydrazine) [1009, 1072, 309] etc.

According to HORITA and McGRATH [600] both an unsubstituted arylalkylhydrazine structure and the presence of a benzene ring are required for the possession of greater brain (than liver) MAO-inhibiting properties. Derivatives lacking the benzene ring or substituted on terminal hydrazine with an isonicotinyl group should inhibit liver MAO more strongly than brain MAO.

Fig. 4
The main MAO inhibitors



Tranylcypromine (SKF *trans* 385, 2-phenylcyclopropylamine).

This drug is a long-lasting inhibitor, approximately 10–100 times more active than iproniazid phosphate [754].

Etryptamine (DL- α -ethyltryptamine; Monase®). DL- α -ethyltryptamine is a reversible inhibitor of MAO, with an *in vitro* activity comparable to that of iproniazid. Concentrations of etryptamine of the order of 5×10^{-5} M and 10^{-4} M inhibit MAO *in vitro* by 26–60 and 50–80%, respectively. *In vivo*, intraperitoneal doses as high as 45 mg/kg are required to cause in rats a 31% inhibition of the brain MAO and a 50% inhibition of the liver MAO. The inhibition obtained with 90 mg/kg doses is 97 and 65%, respectively. On the other hand, doses of etryptamine as low as 1.25 mg/kg seem to enhance the increase of brain 5-HT observed following administration of 5-HTP. Etryptamine does not block 5-HTP decarboxylase, while consistently inhibiting pseudocholinesterase [515, 515b].

N-Benzyl-N-methyl-2-propynylamine (A 19120).

This is a structurally unique long-acting MAO inhibitor which is approximately as active as nialamide and harmaline, and 5–10 times more active than iproniazid in causing a 50% inhibition of the rat liver mitochondrial MAO [1191].

Amphetamine. It has been known for a long time that amphetamine is a moderate inhibitor of MAO. HOPE and SMITH [596] have now demonstrated that D-amphetamine is 7 times as potent as L-amphetamine as an inhibitor of brain MAO.

Harmala alkaloids. *Harmine* and *harmaline* are very potent inhibitors of MAO both *in vitro* and *in vivo*, being more than 100 times as effective as iproniazid, and approximately 5 times as effective as β -phenylisopropylhydrazine. They are the main representatives of the short-acting or reversible MAO inhibitors, which are opposite to the previously discussed long-acting inhibitors. Whereas a single dose of iproniazid or β -phenylisopropylhydrazine produces MAO inhibition *in vivo* lasting up to 7–10 days [598], harmine causes an appreciable block for only a few hours [573, 1230].

PLETSCHER and BESENDORF [947] first observed that pretreatment with harmaline inhibits in rats and mice the increase in brain 5-HT and noradrenaline usually produced by iproniazid. HORITA and McGRATH [599] could confirm the protective action of harmine towards β -phenylisopropylhydrazine, but could not demonstrate any interaction between harmine and SKF-385. In fact, whereas in rats treated with both harmine and β -phenylisopropylhydrazine block of MAO activity was of short duration, as with harmine alone, in rats treated with both harmine and SKF-385 block of MAO activity showed the usual SKF-385 duration curve. It is suggested that whilst harmine, iproniazid and β -phenylisopropylhydrazine may act at the same site on the enzyme, the mechanism of MAO inhibition produced by SKF-385 may be quite different from that of the two other irreversible inhibitors.

Pyridine aldoxime dodecyl iodide (PAD), a known reactivator of cholinesterase, is a potent inhibitor of MAO *in vitro*. *In vivo* MAO block is masked by other predominant actions [823b].

5.56 Biochemical effects of MAO inhibition

The main biochemical effects resulting from MAO inhibition are as follows:

a) Rise in the concentration of free amines in tissues and blood. This rise is observed for strictly endogenous amines, for amines originating in the organism from exogenous precursor aminoacids and, to a lesser degree, for exogenous amines. Obviously, the increase in free amine concentration is strictly dependent upon the relative importance of oxidative deamination in the metabolism of the single amines: block of MAO is highly effective for amines mainly attacked by MAO, much less so for amines having at their disposal alternative metabolic pathways.

The biochemical effects of MAO inhibitors on the amine content of the CNS will be discussed in detail elsewhere. Outside the brain, the most remarkable effect of MAO block on endogenous amines may be observed in blood platelets, the 5-HT content of which is augmented up to 150% in rabbits [942] and 75–100% in man [1115, 792] following iproniazid administration. MAO inhibitors, on the other hand, have little effect on the level of endogenous 5-HT in the gastro-intestinal tract and in other peripheral depots.

Considerably more striking are the effects produced by MAO inhibitors on the tissue levels of amines originating from precursor aminoacids. In fact, the increase in the amine levels obtained with the aminoacid plus iproniazid is

considerably greater than that obtained with the aminoacid alone. To give some examples: (α) 5-HT values increased in the rat blood from 1.65 μg (5-HTP alone) to 3.4 $\mu\text{g}/\text{ml}$ (5-HTP + iproniazid), from 1.4 μg (5-hydroxy-N-acetyl-TP alone) to 2.4 $\mu\text{g}/\text{ml}$ (aminoacid + iproniazid), from 1 μg (5-acetoxy-N-acetyl-TP alone) to 2.8 $\mu\text{g}/\text{ml}$ (aminoacid + iproniazid); in the rat kidney from 11, 11 and 23 μg (the above aminoacids alone) to 33, 72 and 108 $\mu\text{g}/\text{g}$ (the above aminoacids + iproniazid), respectively; (β) 4-HT values increased in the rat liver from 10 μg (4-HTP alone) to 40–70 $\mu\text{g}/\text{g}$ (4-HTP + iproniazid), in the rat kidney from 50 to 110 μg , in the rat heart, lung and uterus from undetectable amounts to 4–6 $\mu\text{g}/\text{g}$ [381, 386, 386b]; finally (γ) tryptamine values increased in the rat liver from 0.2 μg (TP alone) to 5.7 $\mu\text{g}/\text{g}$ (TP + iproniazid) [574].

b) Rise of urinary amines, either free or conjugated with glucuronic and sulphuric acid.

Rise of free amine is particularly striking for tryptamine, which is metabolized almost entirely by MAO and much less so for the hydroxyindolealkylamines which have at their disposal alternative metabolic pathways.

In the case of tryptamine, pretreatment with iproniazid and other MAO inhibitors increased the normal daily urinary excretion of the free amine from 0.6 to 10 μg in guinea-pigs [574] and from 30–110 μg up to 1000 μg in man [1130], raised by more than 100% the recovery, as free amine, of exogenous ^{14}C -labelled tryptamine [1063] and, finally, produced a huge increase of urinary free tryptamine in guinea-pigs given a L-tryptophan load [574].

As already stated, MAO blockade produces inconstant or undetectable increase in the urinary concentration of free hydroxyindolealkylamines, while consistently augmenting the concentration of their sulphates [250] and glucuronides [1287, 1291d].

c) Decrease in blood, tissue and especially urine of the indoleacetic acids resulting from the oxidative deamination of the corresponding amines. Following iproniazid administration to rats, the basal urinary excretion of 5-HIAA falls by 20–30% [101], and the recovery of exogenous hydroxytryptamines or precursor aminoacids as 5-HIAA falls by 75–85% for 5-HT and bufotenine [386d, 270], by 75% for 4-HT, by 85% for 5-HTP, and by 95% for 4-HTP. Even in rat blood and tissues, 5-HIAA and 4-HIAA display a 2- to 6-fold decrease [386, 386b].

Several biochemical methods have been recommended for screening the intensity and duration of action of MAO inhibitors in animals and man. The most simple and reliable of these methods are based on the capacity of MAO to increase the urinary level of free tryptamine [1129, 1130] and to decrease the excess urinary excretion of 5-HIAA produced by oral or parenteral administration of an arbitrary dose of 5-HT [1127, 1187].

Both methods are excellent, provided that one keeps in mind that they are adequate for the demonstration, and even more the quantitation, of MAO inhibition only outside the CNS. In fact, block of liver, kidney or lung MAO does not necessarily parallel block of brain MAO, owing to the different capacities of different drugs to penetrate into the CNS [941, 600].

It has already been emphasized that numerous biogenic amines are substrates for MAO. It follows that in the living organism MAO inhibitors may block the oxidative deamination of all these amines, provoking their accumulation in tissues and blood as well as the compensatory engagement of other inactivating enzyme systems. Thus, the interference of MAO inhibitors is not limited to the metabolism of a single amine, but extends to that of all available amines. The picture of MAO block is further complicated by the fact that it may vary remarkably from one animal species and one tissue to another, depending on numerous conditions such as relative richness of tissue in different amines, turnover rate of the amines, easy availability of alternative pathways for the inactivation of the amines, organ specificity of MAO inhibitors, etc.

For a more complete critical discussion of the positive and negative aspects of the use of MAO inhibitors the reader is referred to pages 312-314. Here it should be stressed that, besides being of inestimable usefulness in the detection and identification of biogenic amines, MAO inhibitors may be valuable tools in the study of the biological effects and biological significance of the amines, provided that one keeps firmly in mind that improperly or incautiously used, these 'nonspecific inhibitors of a relatively non-specific enzyme' [648] may also lead to confusing and negative results.

5.6 Oxidation and rupture of the indole ring

Only a part of the administered hydroxyindoles may be recovered in urine as metabolites still bearing a free or conjugated hydroxy group in the indole ring. Another part is present as coloured derivatives or pigments of unknown constitution, and still another part is undetectable with the colour reactions specific for indoles. This last part must have undergone profound alterations in the body probably involving rupture of the indole ring.

Enzyme systems and/or non-enzymic catalysts leading to an opening of the indole ring and thus to a complete degradation of the compounds have been poorly investigated. More information is available concerning enzymes which oxidize or hydroxylate the indole nucleus, often together with the side chain causing, as a final result, the formation of pigments.

5.61 Tissue enzymes

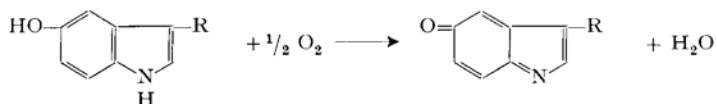
Crude preparations of mitochondrial amineoxidase, which produce the oxidative deamination of hydroxyindolealkylamines to the corresponding aldehydes and then to the indoleacids, are also rich in an enzyme system which oxidizes the aldehydes to pigments [1286]. Although this enzyme may be responsible for the ring oxidation of an important portion of hydroxyindoles, it seems highly probable that other oxidizing systems are active in tissues. One of them, in the gill plates of *Mytilus edulis*, has been described by BLASCHKO [130, 133, 133b].

The *Mytilus hydroxyindole oxidase* is cyanide sensitive and is not inhibited by iproniazid. Good substrates for it are 4-, 5-, 6- and 7-hydroxyindoles (amines,

their precursor aminoacids and 5-HIAA), whereas tryptamine is not attacked. Yellowish-brown or yellowish-orange colours are formed by 5-, 6- and 7-hydroxyindoles, and a blue colour by 4-hydroxyindoles.

It has been suggested that in the enzymatic reaction of the 4-hydroxyindoles an *o*-quinonoid is formed, and that oxidation of the 5- and 6-hydroxyindoles may lead to the formation of *p*-quinones [132, 133].

Of course, it is also possible that true pigments may arise from the oxidation of hydroxyindoles by this enzyme.



5.62 Blood enzymes

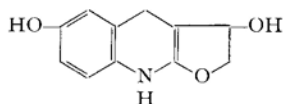
Ceruloplasmin, another hydroxyindole oxidase, has attracted particular attention in recent years as the enzyme mainly responsible for the oxidation of 5-HT and related hydroxyindoles in plasma. The reaction catalysed by ceruloplasmin is believed to yield first a quinone imine derivative which, by further oxidation or hydroxylation, enzymatic or non-specific, could result in compounds structurally related to adrenochrome [953], or even in dark coloured compounds of high molecular weight [1041].

Like gill plate oxidase, ceruloplasmin acts not only on 5-HT, but also on 4-HT, 7-HT and 6-HT, thus demonstrating that its substrate specificity is not restricted to indoles carrying an hydroxy group at C-5 [728].

However, 4-, 5-, and 7-HTP and 5-hydroxyindole were not attacked by caeruloplasmin, which shows that the substrate specificity of the mammalian oxidase is somewhat more restricted than that of *Mytilus* oxidase [133b]. The pH optimum for the oxidation of 5-HT by purified human ceruloplasmin is 4.5-5. The oxidase is strongly inhibited by cyanide and azide ions [290] as well as by sodium metabisulfite, ascorbic acid, glutathione, dimercaptol and MAO inhibitors [795, 1344b].

The 5-HT derivatives isolated by BRACCO *et al.* [158] following incubation of the amine with mammalian serum or plasma are similar to those postulated by PORTER *et al.* [953].

The Italian investigators suggest for their compound FC 23 the structural formula:



Oxyhaemoglobin, especially when undergoing denaturation, may represent another oxidizing agent for 5-HT and other hydroxyindoles. The reaction, which may be completely inhibited by carbon monoxide and partially by ascorbic acid, leads to pink coloured products lacking fluorescence [992, 741, 136].

5.63 Non-enzymic catalysts

Oxidation of 5-HT, 5-HTP and 5-HIAA to black pigments may be produced catalytically *in vitro* by various metal salts. Copper is the most active catalyst of 5-HT oxidation, vanadium and niobium of 5-HTP oxidation [794].

The chromatographic reaction products resulting from the non-enzymatic oxidation of 5-HT by cupric ions are apparently identical with those produced in the enzymatic oxidation of 5-HT by human serum [795].

Oxidation of 5-HT by molecular oxygen or AgNO_3 under specified conditions leads to the formation of coloured products and eventually an insoluble pigment resembling melanin. Of several chromatographically apparent intermediates in this process, one has been isolated by ERIKSEN *et al.* [363] and tentatively identified as a dimer resulting from the dehydrogenative coupling of two 5-HT molecules.

Resolution of the products from U.V. irradiated 5-HT solutions on paper chromatograms showed the presence of formylkynuramine, kynuramine and a pigment-like material [533].

It is very difficult to say how much endogenous or exogenous 5-HT undergoes rupture of the indole ring or is oxidized to pigments. According to the data presented by McISAAC and PAGE, who used ^{14}C -labelled 5-HT, and to results obtained in this laboratory with 5-HTP, the percentage of 5-HT which is no longer detectable in rat urine with the colour reaction characteristic for the indole nucleus is approximately 30 to 50%.

It should be kept in mind in this respect that McISAAC and PAGE [810], and KEGLEVIC *et al.* [640] were able to find in rat urine only 60–70% and 36–82%, respectively, of the radioactivity possessed by the administered ^{14}C -5-HT.

5.7 Excretion of unchanged indolealkylamines

5.71 Urinary excretion

Very small amounts of endogenous indolealkylamines escape enzymatic attack to be eliminated unchanged in urine.

The daily urinary excretion of 5-HT has been found to range in healthy human subjects from 20 to 720 μg [714, 301, 991], that of tryptamine from 20 to 120 μg [301, 991, 1129].

The urinary excretion of 5-HT increases, of course, after administration of exogenous 5-HT or 5-HTP. However, whereas in the first case the small urinary 5-HT (in rats barely 0.6–2% of the subcutaneously administered dose, in mice 4%) [8, 386, 1291 d] actually represents the portion of 5-HT escaping enzymatic attack after circulating, in the latter case, the more abundant 5-HT (up to 20% of the administered L-5-HTP) [1224, 386] is mainly of renal origin, as it derives from the excretion of the 5-HT produced by the decarboxylation of 5-HT within the renal tissue itself [386].

Similarly to 5-HT, varying amounts of exogenous tryptamine (0.8%), bufotenine (7–29%), 4-HT, psilocybin (10–12%), 6-HT (20%) and 7-HT also may be recovered unchanged in urine [1063, 386, 489b].

Following administration of L-tryptophan, 5-OH-N-acetylTP, 5-acetoxy-N-acetylTP and 4-HTP, the urinary excretion of the corresponding amines is less than 0.02% [574, 868b], 4–6%, 4% and 8–20%, respectively, as referred to the L-aminoacid [381, 385].

5.72 Excretion in the faeces

McISAAC and PAGE found that faeces of rats given ^{14}C -5-HT contain 3 to 5% of the radioactivity administered, and faeces of rabbits only 0.15–0.16%. The origin (intestinal or biliary excretion?) and nature of the radioactive material in stools have not been further investigated [810].

6. Peripheral Physiological and Pharmacological Actions of Indolealkylamines

6.1 Action on the systemic blood pressure

The writer's [364] conclusion that '5-HT is neither a pure hypertensive nor a pure hypotensive agent but, according to the dose, the route of administration, the anaesthetic used, the neurogenic vaso-constrictor tone, the general conditions of the cardio-vascular apparatus and, above all, the animal species, 5-HT can elicit hypotensive, hypertensive or mixed responses' still maintains its full validity.

Dog. It has been ascertained that the pressure response of dogs to parenteral 5-HT depends upon the type of anaesthesia and, still more, upon the dose and the route and rapidity of 5-HT administration.

Rapid intravenous injection or rapid injection into the pulmonary artery [755] of relatively high doses of 5-HT (25–200 $\mu\text{g}/\text{kg}$) produces a prevalent hypertensive response with increase in the systemic vascular resistance [755, 119, 601, 614, 3].

Rapid intravenous injection of low doses of 5-HT or slow intravenous injection, or infusion into the veins or the right atrium of higher doses of 5-HT constantly produces a drop in mean systemic blood pressure and a decrease in systemic resistance [1073, 1014, 1013, 803, 804, 889, 285]. At an infusion rate of 60 $\mu\text{g}/\text{kg}/\text{min}$ the pressure fall is 28% and the decrease in systemic resistance 55% [1014].

The same vasodilator and hypotensive effect is obtained when 5-HT is given by rapid injection into the portal vein, instead of into the femoral vein [90]. It would be interesting to confirm the statement of HOTOVY and ROESCH [601] that, in sharp contrast to what happens in unanaesthetized dogs and in dogs anaesthetized with chloralose, rapid intravenous injection of 5-HT in dogs anaesthetized with barbiturates or thiobarbiturates produces a fall of blood pressure and a decrease of peripheral resistances.

Dogs premedicated with reserpine respond to infusion of 4 $\mu\text{g/kg/min}$ 5-HT with a gradual additional fall in blood pressure over the reserpine-induced hypotension [284]. The hypertensive effect of 5-HT given by rapid intravenous injection is, on the contrary, either antagonized [1070, 1071, 1017] or enhanced [1070, 1071] by reserpine pretreatment, according to experimental conditions.

Contrary to expectations, pretreatment with MAO inhibitors (iproniazid, β -phenylisopropylhydrazine, harmine and others) has been found to decrease the cardiovascular effects of 5-HT [503].

Hypertension produced by tryptamine is potentiated by prior treatment with iproniazid or, much more effectively, with iproniazid plus reserpine [191].

The pressor effects of 5-HT (30–50 $\mu\text{g/kg}$) are antagonised potently by LSD and methysergide (UML) in doses as low as 10–20 $\mu\text{g/kg}$ [414] and moderately by BAS, given in high doses (4–8 mg/kg) [614].

In contrast to LSD and UML, BOL was found to exhibit weak and in-constant activity towards the cardiovascular responses to 5-HT [1034].

Contrasting with the predominant hypertensive effect of bufotenine, psilocybin (0.05–0.1 mg/kg, i.v.) produces in the dog a moderate but sustained fall of blood pressure, tachycardia and active reduction of the kidney volume [1279].

The opinion has been repeatedly advanced that 5-HT hypertension may be mediated through the liberation of catecholamines from the adrenal medulla and the vascular wall [1057, 1058, 119], and 5-HT hypotension through liberation of histamine [1057, 889]. However, results obtained from reserpinized animals cast considerable doubt on the actual importance of an eventual catecholamine release.

Cat. Hypotension is the predominant blood pressure response to intravenous 5-HT in the intact cat. More rarely, a mixed response or a pure hypertensive reaction may be seen. In spinal animals 5-HT nearly always produces a rise of blood pressure.

Since hypotension may be reduced or abolished by vagotomy, by cooling the vagi to 1.5–2° or by intravenous administration of local anaesthetics, it has been suggested that it is mainly of reflex origin. Impulses producing reflex peripheral vasodilation and reflex bradycardia are carried to the centres by afferent vagal fibres [677, 1276].

The blood pressure rise produced by 5-HT in spinal cats, in its turn, is considered due not only to a direct constrictor effect on the vascular smooth muscle, but also to an increase in the heart rate and to stimulation of the adrenal medulla [1277].

Cardiovascular responses to 5-HT in the cat are only slightly and irregularly antagonised by BOL and LSD [1034, 1277]. Pretreatment with iproniazid (2 $\times$ 50 mg/kg) or reserpine (2 mg/kg) has failed to produce pressure changes different from those seen in untreated animals; pretreatment with 2-naphthylguanidine (100–500 $\mu\text{g/kg}$), on the contrary, causes 5-HT to elicit a rise in blood pressure instead of the usual fall [393].

All the other indolealkylamines so far tested produce a predominantly hypertensive response in the intact animal: this is true for psilocybin, psilocin, 4-HT, bufotenine and bufotenine O-phosphate. None of these amines is as active as 5-HT [1279, 1280]. Intravenous 5-HTP, in doses of 2.5–5 mg/kg elicits a moderate fall of blood pressure, which lasts 15–20 minutes; the same dose of 4-HTP produces a more pronounced rise of blood pressure, lasting 30–50 minutes [382].

In pithed cats and dogs, as well as in animals with ganglionic block both 5-HT and bufotenine produce elevation of blood pressure. O-methylation reduces the vasopressor activity of 5-HT but greatly enhances that of bufotenine; N-acetylation of 5-methoxytryptamine destroys its activity on blood pressure [474c].

Rabbit. It is probable that the systemic hypotension which is regularly seen in the rabbit following intravenous 5-HT (25–250 μ g/kg) is in part dependent upon pulmonary vasoconstriction and consequent reduction of cardiac output [707].

Rat. Confirming previous reports, SALMOIRAGHI *et al.* [1033] found that intravenous injections of 1–2 μ g 5-HT into unaesthetized rats produce a small transient fall in arterial pressure (von Bezold-Jarisch-like effect) succeeded by a small pressor response, followed in turn by marked and prolonged hypotension. Vagus section abolishes the Bezold-Jarisch-like effect; neither vagus section nor inactivation of the carotid sinuses modifies the prolonged depressor response.

After ganglionic blockade or pithing, 5-HT elicits only large pressor responses. LSD and BOL in high doses effectively antagonize pressor-depressor responses to 5-HT in rats [1034].

In contrast to 5-HT, bufotenine and psilocybin produce a pure rise in blood pressure not only in rats with ganglionic blockade but also in normal rats. In addition, the pressor response is considerably more sustained than with 5-HT. BOL is again a good antagonist [489b].

Intravenous 5-HT renders the rat mesoappendix hyperreactive to locally applied adrenaline when given at doses of 150–250 μ g/kg, but depresses vascular reactivity to adrenaline when given at higher doses [1357].

Chicken. Intravenous 5-HT in chickens produces a fall of blood pressure. The minimum effective dose is 10 μ g/kg, and maximal effects are elicited by 200 μ g/kg. Tryptamine is 3–5 times less active than 5-HT. 3 mg/kg BOL effectively antagonizes the vascular action of 5-HT [956, 708].

The depressor response is not significantly affected by vagotomy, physostigmine or mecamylamine; it is on the contrary reversed by atropine and either blocked or altered by antihistaminic drugs. On the basis of these data BUNAG and WALASZEK [190b] suggest that the major factor responsible for the depressor response to tryptamine and 5-HT in chickens may be a liberation of histamine.

Man. Single intravenous injections of 5-HT (0.2–4 mg) produce both in normotensive and hypertensive subjects variable responses probably dependent,

to some extent, on the dose and on the rapidity of administration of the drug [763, 977, 50, 19, 137, 591]. Depressor responses occur more often after doses less than 0.3 mg; biphasic responses (brief fall and then overshoot) become more frequent over 0.3 mg; pressor responses (increase in both systolic and diastolic pressures, eventually followed by a brief drop) towards 1 mg and more [591].

The blood pressure effects of 5-HT are not prevented by prior administration of hexamethonium, Regitine®, atropine, antihistamines or reserpine [977, 591]. BAS in intravenous doses greater than 45 mg often blocks the pressor but not the depressor effects of 5-HT [591].

Following infusion of 5-HT into a vein [591, 716] or into the right heart [523], at a rate of 10–1000 $\mu\text{g}/\text{min}$, systemic arterial pressure displays only minor and variable variations, while, at the highest dosage levels, systemic vascular resistance decreases [523].

Possible side-effects of intravenous infusion of 5-HT in man are an increase in local venous pressure with possible reflux of blood into the afferent vessels [1170], burning pain along the course of the vein which may become prominent, firm and tender; heaviness or tingling in the extremities; pressure in the head and eyes; nausea; cough; shortness of breath; abdominal cramps, and, occasionally, blurred vision, sweating, headache and excessive salivation [591, 716, 891].

Thrombosis of the infused vein is exceptionally frequent (40 and 90%) when 5-HT is infused at a rate of 400 and 800 $\mu\text{g}/\text{min}$ [1170].

6.2 Action on special vascular areas

Pulmonary vessels. REID [985] first observed that intravenous injection of 5-HT in the cat produces, as a consequence of an increase in the pulmonary resistance, a rise of pressure in the right ventricle and in the pulmonary artery, accompanied by a fall in the pulmonary vein.

These observations have been confirmed and extended by numerous research workers [803, 804, 866, 285].

In the dog 5-HT has proved to be a potent constrictor of the pulmonary vessels, even in small doses, such as 1–5 $\mu\text{g}/\text{kg}$ by single injections into a vein or into the pulmonary artery or 1–3 $\mu\text{g}/\text{kg}/\text{min}$ by i.v. infusion [857, 858, 150]. The action of 5-HT on this vascular area exceeds that of angiotensin [224] and of all other biogenic amines, including catecholamines [994, 150, 492b].

Infusion rates as low as 8 $\mu\text{g}/\text{kg}/\text{min}$ result in a two-fold increase of pulmonary arterial pressure in the dog, and in a 55% increase in the difference of pressure between the pulmonary artery and the left atrium [857, 858]. Infusion rates of 60 $\mu\text{g}/\text{kg}/\text{min}$ produce a 300% increase in the mean pulmonary pressure. Calculated pulmonary resistance increases up to 500% of control levels [1013, 1014].

Due to pulmonary vasoconstriction, the pulmonary blood volume [807] and arterial oxygen saturation [1014] usually are reduced considerably during 5-HT infusion.

Although the principal site of the increased resistance to flow through the lungs appears to be in the pulmonary arterioles [625] or precapillary vessels [1100], pulmonary venules also are constricted by 5-HT, especially when given in large doses [625, 1100].

In fact, if 5-HT produces an increase in the pressure gradients between the pulmonary artery and pulmonary artery wedge pressures [625, 488, 489], as well as a reduction in the lung weight (signs of increased arteriolar constriction), it also causes an increase in the pressure gradients between the pulmonary artery wedge pressures and the left atrial pressure [625, 488], as well as an increase in venular pressure, as measured directly [488, 39] (signs of increased venular constriction).

High doses of 5-HT (0.2–3 mg) injected into the dog pulmonary artery produce moderate to severe pulmonary oedema with occasional evidence of left heart failure. Without excluding other mechanisms [625], it seems probable that the change in capillary permeability provoked by 5-HT is due to a direct action of 5-HT itself on the pulmonary capillary wall.

The increase in pulmonary vascular resistance caused by 5-HT may be reduced in the dog by augmenting the pulmonary blood flow as well as by raising the pulmonary venous pressure [1016].

Promethazine, BOL and LSD (0.4–0.6 mg/kg) effectively antagonize the constrictor action of 5-HT on pulmonary vessels [994]. Vasoconstriction produced by continuous infusion of 5-HT is in part also antagonized by acetylcholine, histamine and ATP [1015].

It is probable that, like other vascular areas, the pulmonary bed presents conspicuous species differences in its sensitivity to 5-HT. In man, for example, doses of 0.8–2.7 $\mu\text{g/kg/min}$ 5-HT base given by i.v. infusion fail to produce any change in the pulmonary artery pressure [549], and similar doses infused into the pulmonary artery elicit only a rise in the mean pulmonary arterial pressure, while the wedge pressure remains unchanged and changes in total pulmonary vascular resistance are inconstant and variable [523].

Similarly, in calves intravenous infusion of 5-HT (0.2 mg/kg/min for 3 minutes) fails to alter significantly pulmonary artery pressure, pulmonary artery wedge pressure, pulmonary vascular resistance and cardiac output, thus demonstrating that in the bovine species, too, 5-HT is not an effective pulmonary vasoconstrictor agent. The systemic blood pressure, on the contrary, decreases significantly [681b].

It is a matter for debate whether 5-HT can initiate systemic vascular reflexes in the pulmonary vessels [994].

Liver vessels. An injection of 15 μg 5-HT into the portally perfused canine liver produces a small increase in the resistance to blood flow through the liver. However, 1 mg 5-HT added to 500 ml of perfusing liquid causes no congestion. Increased resistance probably occurs in the portal vessels before the hepatic artery and the portal vein join [17].

In substantial accordance with these findings, SHOEMAKER *et al.* [1105b] observed that in unanaesthetized dogs hepatic plasma flow decreases to appro-

ximately half the control value 1 to 2 minutes after the injection of 0.1 to 0.2 mg/kg 5-HT base into the femoral vein. Concomitantly there is an increase in portal and hepatic venous potassium and a fall in portal and hepatic venous glucose levels.

Vessels of the placenta. 5-HT injected into the umbilical artery of the perfused human placenta displays a potent constrictor action which is about 10–20 times stronger than that of adrenaline and noradrenaline.

Doses of 0.3–3 μ g provide moderate vasoconstriction; doses of 5–10 μ g often cause complete closure of the vessels. Tryptamine is 10–20 times less active than 5-HT.

The action of 5-HT is enhanced by cocaine and antagonized by dihydroergotamine, LSD, tryptamine, yohimbine and chlorpromazine. Ephedrine, iproniazid and reserpine are ineffective [353, 38].

The results obtained on a non-innervated preparation are of particular interest, as they speak decidedly in favour of a direct action of 5-HT on the vascular smooth muscles.

RODEGRA *et al.* [989] suggest that 5-HT liberated from blood platelets may participate in the spontaneous closure of the umbilical artery after birth.

Coronary vascular bed. In anaesthetized dogs, 5-HT infused into the right atrium at a rate of 8 μ g/kg/min produces a 20% increase in coronary blood flow, a 27% decrease in coronary vascular resistance and a 31% increase of coronary sinus oxygen content. Cardiac oxygen consumption and CO₂ production increase, while mechanical efficiency of the left ventricle falls by 3% [803, 804, 285]. Similar results have been obtained following injection of 5-HT (1–30 μ g) directly into the left main coronary artery: there is a prompt fall in coronary vascular resistance, with no change in myocardial oxygen consumption [522b].

Thus, in confirmation of previous work [1085], 5-HT appears to act as a powerful coronary dilator increasing the oxygen supply to the myocardium without increasing the ventricular work.

Musculo-cutaneous vessels. Careful experiments have been carried out in the dog and in man. 5-HT infused into the brachial artery of the foreleg of anaesthetized dogs at a rate of 4–5 μ g/min does not significantly change the total resistance of the foreleg vessels, but it does increase large artery resistance and, sometimes, vein resistance and does decrease small vessel resistance. This means that large arteries and veins are constricted by 5-HT, whereas the small vessels are dilated. HADDY *et al.* [528, 529, 531] are of the opinion that 5-HT-induced segmental resistance changes occur through active mechanisms. They further suggest that a function of 5-HT may be that of influencing volume distribution of blood within various segments of the circulatory system as well as influencing water and salt distribution between intravascular and extravascular compartments.

Besides small vessel dilation, 5-HT also produces oedema during prolonged infusion, as a consequence of increased capillary hydrostatic pressure provoked by venous constriction [532].

Because of the directionally opposite changes in segmental resistance it

produces, 5-HT is capable of antagonizing extremes of vascular tone induced in the dog foreleg by neurogenic means. Vasodilation produced by denervation of the foreleg is antagonized by the constriction of the large vessels caused by 5-HT; vasoconstriction produced by bilateral vagotomy is counteracted by the small vessel dilation similarly caused by 5-HT. A similar antagonism is more difficult to demonstrate when tone is varied by humoral means [530].

In human subjects 5-HT infused into the brachial artery in doses of 1–16 $\mu\text{g}/\text{min}$ causes a reduction of blood flow through the forearm (30% with 1 $\mu\text{g}/\text{min}$, 50% with 4 $\mu\text{g}/\text{min}$ and 70% with 16 $\mu\text{g}/\text{min}$) as well as through the hand, with a concomitant volume increase (due to oedema formation) and a marked flushing of the skin. In the forearm the fall of flow is preceded by a transient increase and, as the infusion continues, cyanosis develops gradually in the skin of the fingers. Small petechiae are occasionally observed some hours after the infusion. Subjective symptoms are a tingling sensation and, with high doses, severe but fleeting pain.

The above phenomena have been ascribed to dilation of capillaries or lower-pressure capacity vessels, mainly responsible for the colour of the skin, and to constriction of arterioles or higher-pressure capacity vessels, mainly responsible for resistance to flow.

These findings and the interpretations of GLOVER, WHELAN *et al.* [988, 497] are in substantial accordance with those of CALI and LOMEO [204, 205, 210, 211] and of MAGALINI *et al.* [763], who similarly found a constrictor action of 5-HT in the arterioles, a dilator action on capillaries and a constrictor action on the veins, together with an increase in the finger volume.

The question remains open whether flushing of the skin should be considered an active phenomenon [204] or a manifestation of passive hyperaemia [137].

Both BOL and sodium salicylate have proved to be specific antagonists of the constrictor response of the forearm and hand blood vessels to intraarterial infusion of 5-HT but whereas BOL displays a direct antagonistic action, sodium salicylate acts only indirectly, possibly through release of, or synergism with, some other substances [496].

Following intravenous administration of 5-HT, changes in human forearm vessels are in many respects different from those observed after intra-arterial injection of the amine. In fact, intravenous infusion of 0.5–1.2 mg 5-HT base/min causes a dilator response of the forearm vessels consisting of a huge initial transient increase in flow, followed by a smaller (20 to 30%) but sustained increase [716]. It has been suggested that the sustained increase in flow probably represents a balance between the direct constrictor action of the drug and a secondary vasodilator effect.

According to BOCK *et al.* [137] both intravenous and intra-arterial 5-HT constantly increase the flow in muscular vessels, while reducing that in the cutaneous vessels.

Intravenous 5-HT produces pain along the course of the infused vein and transitory elevation of local venous pressure. The local venoconstrictor effect is appreciable with doses of 5-HT as low as 1.5 μg given within 5 minutes [763].

5-HT applied topically on the vessels of the cheek pouch of the hamster produces intense constriction, the threshold concentration being $0.01\text{--}0.1\text{ }\mu\text{g/ml}$ [14].

Similarly the drug displays considerable vasoconstrictor and haemostatic activity both in animals and human beings [861, 265, 433] when put into direct contact, in sufficient concentrations ($0.02\text{--}0.1\%$ solution) with bleeding surfaces. These experimental results represent the basis of the recommended therapeutic use of 5-HT in the control of bleeding from small vessels during surgical operations [316, 753, 892, 1018, 1044, 1202, 1258, 73].

Of course, management of peripheral bleeding with 5-HT would be rational only if the advantages of 5-HT over the classical haemostatic drugs were definitely established. This, however, has not yet been done.

Vessels of the kidney. See pages 218-223.

Vessels of the hind leg of the rat. 5-HT is about twice as active on the hind leg vessels as on the abdominal aorta of the rat [1181]. In rats made hypertensive through clipping of one renal artery, the vasoconstrictor action of 5-HT ($0.5\text{ }\mu\text{g/ml}$) on the perfused hind leg is the same as in control normotensive rats, but in hypertensive animals the amine counteracts oedema formation following perfusion more powerfully [1213].

Vessels of the mouse ear. They were studied using a technique of freeze fixation which permits stereomicroscopic observation of the terminal vascular bed in a life-like state. Single i.v. doses of 5-HT produce (a) extravasation of parenterally administered Evans Blue (after 50 or more $\mu\text{g/kg}$), (b) vasodilatation occurring directly after doses of $50\text{--}250\text{ }\mu\text{g/kg}$ of following an initial period of vasoconstriction after larger doses, and (c) vasoconstriction, increasing in duration with dose (minimum $250\text{ }\mu\text{g/kg}$). UML, 10 mg/kg , partially inhibits these effects. Local administration of 5-HT produces comparable results. 5-HT is 20 times more potent than histamine in provoking vasodilatation and dye escape [1178c].

Vessels of the hind leg of the frog. The outflow rate of the perfusion liquid from the frog hind leg shows rhythmical variations indicating an alternation of periods of vasoconstriction and periods of vasodilation. This spontaneous rhythmical activity is stimulated, or evoked if lacking, by 5-HT at concentrations from 10^{-9} to 10^{-4} . Tryptamine is about 100 times less active than 5-HT [1341].

Vessels of the retina. Single intravenous doses of 4 mg/kg 5-HT produce a transient spasm of the retinal arteries in the rabbit; repeated doses also cause morphological changes consisting of a thickening of the muscular layer of the retinal vessels [844].

Brain vessels. It has been repeatedly observed that intravenous 5-HT produces in rabbits [64], cats [771] and dogs [1059] a blanching of the exposed brain cortex.

CASELLA *et al.* [219] have now tried to follow more exactly the changes in cerebral blood flow by means of thermoflissimetric elements applied to the cortex of the temporal lobe. 5-HT is given intravenously in doses of $0.2\text{--}4\text{ mg/kg}$.

In the rabbit 5-HT causes a moderate drop in cerebral blood flow which lasts 5 to 6 minutes and closely follows the fall of the systemic blood pressure.

In the cat a first phase of decreased blood flow is followed by an increase in flow, which is independent of any change in systemic blood pressure and possibly is due to regional vasodilation.

It is evident that further studies are needed to solve the important problem of whether 5-HT is capable, especially when given by intra-arterial injection, of provoking general or regional changes in the calibre and/or permeability of cerebral blood vessels.

Aorta strips. VACEK [1237] finds the susceptibility to 5-HT of rabbit aorta strips to be considerably below the normal in animals made hypertensive by DOCA and NaCl. The cause of the phenomenon is supposed to be in secondary changes in the aorta wall.

Although the bulk of experimental evidence is rather against than in favour of it, the theory that 5-HT is one of the factors participating in the physiological control of vascular tone and hence of the systemic blood pressure is reinvigorated from time to time. Among the more recent sustainers of this theory are GORDON and co-workers [506, 508, 507].

On the basis of synergistic and antagonistic actions manifested by 5-HT towards the adrenalines, the above investigators suggest that 'a biological role of serotonin lies in its negative interaction with the adrenalines' and that 5-HT may decrease small vessel tone 'through its capacity to antagonize the vasoconstrictor action of noradrenaline on small vessels.'

It should be noted that no negative interaction of 5-HT with noradrenaline could be demonstrated by BROSMAN [178] on the kidney vessels, that the doses of 5-HT used by GORDON *et al.* in most of their experiments were quite unphysiological and finally that results obtained in a single animal species have been unduly generalized.

Actually, it is by no means possible to exclude the possibility that 5-HT may intervene, in particular vascular areas and in particular animal species, in the 'physiological' regulation of tone and calibre of blood vessels, but this does not seem sufficient to allow us to draw conclusions of general value.

6.3 Action on capillary resistance

It has been repeatedly claimed that 5-HT increases capillary resistance both in man and in experimental animals and, on the basis of this claim as well as of the circumstance that 5-HT is contained in large amounts in blood platelets, the drug has been considered to be one of the physiological factors involved in the maintenance of normal capillary resistance and has been recommended in the treatment of morbid syndromes characterized by abnormal capillary fragility.

Actually, experimental and clinical results obtained in this field are often contradictory, and the bulk of evidence is against the hypothesis of a relationship between 5-HT and capillary resistance.

a) *Action of endogenous 5-HT on capillary resistance.* Animals and human beings whose platelets have been nearly completely depleted of their 5-HT

through acute or chronic administration of reserpine do not show any significant change in capillary resistance [554, 1342]. The same is true for rats in which biosynthesis and metabolism of 5-HT is halved by removal of the large intestine [1343].

b) *Action of exogenous 5-HT on capillary resistance.* In subjects with normal capillary resistance [208, 445, 19], there is no change following single or repeated intravenous or intramuscular doses of 2–5 mg 5-HT.

On the other hand, in subjects showing an abnormal capillary fragility, petechial count appears to be consistently reduced following repeated doses of 5-HT. This has been observed in elderly people [1, 72, 445], in children [699] and in tuberculous patients [1004]. Increase of capillary resistance sometimes persists for days and weeks [72], and this frankly casts some doubt upon the interpretation, if not the credibility, of experimental results.

According to BRACCO *et al.* [158] the possibility should be kept in mind that 5-HT is active on capillary resistance and permeability mainly after its transformation by plasma enzymes into oxidation derivatives.

In fact, two of these derivatives have been found to possess a positive action on capillary resistance in guinea-pigs, both normal and with C-avitaminosis, as well as in adrenalectomized rats (active dose 50 μ g/rat), and to antagonize also experimental inflammation provoked by croton oil or formalin.

c) *Therapeutic trials of 5-HT in haemorrhagic syndromes ascribed to increased capillary fragility.* 5-HT has been used, especially in Italy, in the treatment of Werlhof's disease and related haemorrhagic syndromes. The doses recommended are 2–4 mg 5-HT base, intravenously or intramuscularly, every 4–8 hours. Unfortunately, the first enthusiastic reports [10, 70, 71, 156, 971] could not be confirmed by more accurate studies [1170, 825, 1355, 1120] which have given only inconstant and fleeting beneficial effects.

It should be added that it is difficult to decide to what extent inconstant and partial normalization of the tourniquet or suction tests seen following 5-HT administration is really dependent on increased capillary resistance rather than on vasoconstriction [1].

Platelets and platelet derivatives have been used profitably in the control of thrombocytopenic states. However, their beneficial effect is not due to their content of 5-HT [1171].

6.4 Role in haemostasis

Following nearly complete depletion of platelet 5-HT provoked by reserpine in animals and man, no significant changes could be observed in bleeding time, clotting time, prothrombin time, prothrombin consumption time, thromboplastin generation tests with platelet dilutions, platelet adhesiveness and clot retraction time [1109, 1110, 1292, 605].

These results make it difficult to defend the thesis that 5-HT in the platelets is important to the organism as a defence mechanism against bleeding.

It is similarly highly improbable that death due to haemorrhage observed in rabbits subjected to a stress procedure plus reserpine administration is attributable, as suggested by JAUQUES and FISHER [617], to depletion of platelet 5-HT.

High, unphysiological doses of exogenous 5-HT are capable of reducing the bleeding time of experimental animals [329, 387, 433]. This has been confirmed in normal human subjects by STEFANINI and MAGALINI [1170] (0.2 mg/kg base, infused over a period of hours) and by BELLA and ROSSI [79], but not by SIEGENTHALER [1120] and ANNONI *et al.* [19] under practically the same experimental conditions.

In patients suffering from haemorrhagic disorders results are again at variance: a reduction of bleeding time has been observed by STEFANINI and MAGALINI [1170] in vascular pseudohaemophilia but not in thrombocytopenic purpura; LEWIS (quoted by ZUCKER) [1355] has had extremely short-lived positive results in thrombocytopenic purpura; finally SIEGENTHALER [1120] has obtained a reduction of the bleeding time only in those cases of essential or secondary thrombopenia (5 cases out of 24) in which it was augmented. These data seem to be sufficient to prove the extremely erratic and inconstant character of the action of 5-HT on bleeding time.

In regard to the possible interference of 5-HT on the clotting mechanism *in vitro*, it has been asserted

- a) that 5-HT (100 $\mu\text{g/ml}$) enhances clot retraction [405, 78],
- b) that 5-HT accelerates the conversion of fibrinogen to fibrin in the presence of thrombin, acting either on fibrinogen or on an antithrombin which blocks the fibrinogen to fibrin reaction [831, 637], and, finally,
- c) that 5-HT (minimum active concentration 400 $\mu\text{g/ml}$) reduces the antithrombin time prolonged by the action of heparin [645].

Apart from the fact that no action of 5-HT on clot retraction could be confirmed by MAGALINI and STEFANINI [762, 765] and that the *in vitro* addition of 5-HT in amounts up to 20 $\mu\text{g/ml}$ failed to alter any of the clotting factors studied by WEINER and UDENFRIEND [1292], it should be emphasized that the above positive results have been obtained with concentrations of 5-HT 100–2000 times greater than those present in human blood, a circumstance which obviously limits their importance.

Despite the very poor experimental evidence supporting the view that 5-HT interferes in haemostasis, the drug has been tried clinically in the control of surgical bleeding [208, 19, 22, 91, 455, 915, 1027], as well as in spontaneous haemorrhages from nearly all organs (uterus and genital tract [79, 23, 355, 230, 395]), kidney and urinary tract [355], gastro-intestinal tube [355, 520], lung [355, 325], meninges [53] etc.), and of the most different etiology [61, 796, 1061].

The results reported often have been satisfactory, but the writer's impression is that not infrequently clinical experimentation has been carried out without the necessary accuracy and critical evaluation. Consequently it is highly questionable whether 5-HT may be considered a useful drug in the treatment of haemorrhage.

6.5 Action on capillary permeability

ROWLEY and BENDITT [1010] first demonstrated that subcutaneous or intradermal injection of doses of 5-HT as small as 0.1–1 μg in 0.05–0.1 ml liquid produces oedema in rats, and that 200 times more histamine is needed to produce oedema of equal severity.

These results were soon confirmed and extended [511, 512], and 5-HT oedema of the rat paw is often employed in the study of 5-HT antagonists and in the evaluation of anti-inflammatory agents. Methods for a quantitation of the serotonin-induced oedema have been proposed by ECKHARDT *et al.* [346] and by UNGAR *et al.* [1236].

An electron microscopy study carried out on the vessels of the rat cremaster, showed that following subcutaneous injection of 0.3–2.2 μg 5-HT into the scrotum there is an evident damage of the cremaster vessels belonging to the venular tree (presence of gaps between the endothelial cells with intact basement membrane), whereas the capillaries are essentially intact [770b].

Of all the substances studied for their inhibitory action on 5-HT oedema the most active are cyproheptadine (ED 50 = 30 $\mu\text{g}/\text{kg}$) [1178b] and some lysergic acid derivatives such as, in this order, UML (1-methyl-D-lysergic acid butanolamide; ED 50 = 13 $\mu\text{g}/\text{kg}$), PML (1-methyl-D-lysergic acid propanolamide), D-lysergic acid butanolamide and LSD (ED 50 = 57 $\mu\text{g}/\text{kg}$), followed by BOL (ED 50 = 196 $\mu\text{g}/\text{kg}$) [909, 910, 235, 236, 330], chlorpromazine (1–25 mg/kg) [235, 236, 1177, 330] and related phenothiazines (methiotrimpezazine 0.25 mg/kg [909]; promethazine 1–10 mg/kg etc.) [511, 235, 236] and by Dibenamine® [909, 235, 236].

Of minor and apparently less constant activity are aminopyrine (200 mg/kg), phenazone (200 mg/kg) acetylsalicylic acid (500 mg/kg), sodium salicylate (400–600 mg/kg) [641], Atophan®, Tofranil®, chloroquine (10 mg/kg), adrenaline, etc. [235, 236, 845, 1195].

Virtually inactive are BAS, ACTH, STH, cortisone, estradiol benzoate, calcium gluconate, phenobarbital sodium and others [235, 236, 845].

General acral swelling (paws and snout) is also observed in rats and hamsters following subcutaneous or intraperitoneal injections of 5-HT [142, 36b].

In sharp contrast to rats and mice, 5-HT has only negligible potency, if any, as a permeability factor in the skin vessels of other species, including man [1150].

In man, for example, intradermal 5-HT is actually capable of producing changes in the calibre of capillaries (generally local erythema) but does not cause any whealing [571, 261]. On the contrary, rather a thickening effect of 5-HT on capillaries could be demonstrated in some cases, e.g. in the instance of wheal produced by histamine [241] or tuberculin injections [838].

The above findings once again emphasize the danger of generalizing results after experimentation in only one or a few species.

6.6 Action on the heart

Heart in situ. Effects of 5-HT on the heart of intact animals are variable depending on animal species and on route and rapidity of 5-HT administration.

In man both rapid intravenous injection [50, 19, 591] and intravenous infusion [716, 523] generally produce an increase in heart rate which precedes pressure changes, suggesting a direct effect on the heart [716]. Although stroke volume is reduced [19], cardiac output rises [523].

In the dog rapid intravenous injection generally produces bradycardia [614, 755, 601, 748], with a rise in cardiac output, whilst intravenous infusion elicits tachycardia, the cardiac output being decreased ($2.5 \mu\text{g/kg/min}$) [866], unchanged ($8 \mu\text{g/kg/min}$) [285, 804] or increased ($60 \mu\text{g/kg/min}$) [1013, 1014] according to the 5-HT dosage.

The main ECG disturbance produced by intravenous 5-HT consists of a negative T wave during the phase of increased blood pressure [1274].

Cats behave substantially like dogs in regard to their response to rapid intravenous administration of 5-HT. Bradycardia is reduced or disappears following vagotomy, cooling of the vagi or intravenous injection of local anaesthetics [677, 1278].

The cardiac actions of 5-HT, in contrast to those of tryptamine, are not potentiated by prior administration of MAO inhibitors [503].

Heart-lung preparation. On the heart-lung preparation of dogs and rabbits, 5-HT is ineffective in doses up to $10\text{--}20 \mu\text{g}$. At doses of $25\text{--}50 \mu\text{g}$ the amine displays on the rabbit heart a stimulant action with increased cardiac output. In the dog, the stimulant effect elicited by $150\text{--}500 \mu\text{g}$ 5-HT is preceded by a short-lasting inhibition [766].

On the heart-lung preparation of rats 5-HT is ineffective in doses up to $2 \mu\text{g/ml}$; larger doses ($2\text{--}35 \mu\text{g/ml}$) cause the appearance of extrasystoles [833c].

Isolated heart. The effects of 5-HT on the isolated perfused rabbit heart have been thoroughly studied by JACOB and POITE-BEVIERRE [615] over a wide dosage range of 5-HT ($2 \mu\text{g}$ to 5 mg of the base). In general three successive phases could be observed:

- a) irregular negative inotropic and chronotropic effect, antagonized by atropine;
- b) positive inotropic and chronotropic effect, which disappears after pre-treatment with reserpine and is considered to be mediated by catecholamines;
- c) negative inotropic effect, resistant to atropine and often accompanied by a tonus increase. This effect is the predominant one for doses of 0.5 mg and more.

With 4 mg and more atropine-resistant arrhythmias develop, consisting of cardiac arrest, atrio-ventricular block, flutter and fibrillation.

BAS proved to be in this preparation an ambiguous 5-HT antagonist, sometimes mimicking rather than counteracting the 5-HT effects.

On the frog heart 5-HT and tryptamine display a very moderate inotropic effect. This is potentiated by iproniazid and isoniazid, probably as a con-

sequence of a decreased penetration of the amines into the muscle cells [191b].

Isolated atrium. The response to 5-HT ($1\text{ }\mu\text{g/ml}$) of cat atria consists of an increase of rate, whereas changes in amplitude of contractions are small and variable; the response of guinea-pig atria consists of a pronounced and regular increase in amplitude of contractions and in rate of beat. In both species the effect of 5-HT seems to be direct, as it is blocked by LSD and remains unchanged after pretreatment with reserpine [1211b].

Effects observed on the isolated rabbit atria are at variance. Whereas LOUBATIÈRES *et al.* [748] could not see any influence by 5-HT ($0.05\text{--}1\text{ }\mu\text{g/ml}$) on the frequency and contractile strength of this preparation, others [729, 1211b] found that the amine ($0.1\text{--}10\text{ }\mu\text{g/ml}$) produced a pure stimulant effect (increase in amplitude of contraction and in rate of beat), or a stimulant effect which was preceded by a short-lived inhibition. Since atria of rabbits pretreated with reserpine show very little or no response to 5-HT, it has been suggested that 5-HT acts on some nervous tissue of this preparation and that the stimulant action of the amine is the result of the liberation of noradrenaline.

Iproniazid does not affect the response to 5-HT, but potentiates the response to tryptamine [191b].

Papillary muscle. On papillary muscle preparations of cats, dogs and rabbits, LOUBATIÈRES *et al.* [748] could not see any increase of the contractile strength at final concentrations of 5-HT ranging from 0.05 to $100\text{ }\mu\text{g/ml}$. LEUSEN and LACROIX [731], on the other hand, have observed a positive inotropic effect on the cat papillary muscle at final concentrations of $1\text{--}100\text{ }\mu\text{g/ml}$. This, however, is sometimes rather short-lived and is often preceded by a very brief depressive phase.

Strips of turtle ventricle. 5-HT in concentrations from 0.1 to $20\text{ }\mu\text{g/ml}$ displays minimal and inconstant effects on contractions and ECG. Concentrations from 50 to $1000\text{ }\mu\text{g/ml}$ are depressant. 5-HT does not alter the toxic effects of ouabain or procainamide [543b].

Hearts of molluscs and crustaceans. The stimulant action of 5-HT on the molluscan heart, first described by ERSPAMER and GHIRETTI [363b] has been confirmed by all subsequent research workers. However, the sensitivity of the preparation and the regularity of its response varies a great deal in different molluscan species [454b].

The response of the heart of *Venus mercenaria*, one of the most commonly used preparations, shows three components: an increase in amplitude, an increase in frequency and an increase in the resting tone of the muscle. The relative importance of these components varies with the concentration. At low to moderate concentrations (10^{-9} to 10^{-6}M) a positive inotropic effect is dominant; at high concentrations (10^{-5}M and more) a large increase in muscle tone. Hearts exposed to high concentrations of 5-HT or other tryptamine analogues for long periods become tachyphylactic to low doses of these substances [521].

Congeners of 5-HT without the 5-hydroxyl group tend to act more slowly and irreversibly as well as less strongly than 5-HT. Methyl substitution also increases the time of action and difficulty of reversal. Bufotenine is the most potent indolealkylamine so far tested on the *Venus* heart, being 10 to 100 times as active as 5-HT [522].

The effect of 5-HT is regularly antagonized by BOL, whereas LSD behaves in a strikingly different way: on the *Cyprina* and *Buccinum* heart LSD acts as a good 5-HT antagonist, but on the *Venus* heart the drug displays a powerful irreversible 5-HT-like action [1296, 1295].

Species differences in the sensitivity of the heart towards 5-HT are even more striking for crustaceans than for molluscs. Among the most sensitive hearts is that of *Cancer borealis*, stimulated by 5-HT concentrations as low as 10^{-8} to 10^{-9} M [1295].

Although considerable evidence supports the view that 5-HT in some lower animals may be an excitatory agent liberated by cardioregulatory nerves [1295, 824], caution should be used in concluding that in all molluscan species and, even more, in all crustacean species 5-HT has a physiological role in the control of cardiac activity.

6.7 Action on the circulation and the function of the kidney

Rat and mouse. The writer's data [364] on the powerful antidiuretic effect of 5-HT in the rat have been fully confirmed and the interpretation that this effect is mainly due to afferent glomerular vasoconstriction has been generally accepted [317, 318].

VAN ARMAN and JENKINS [1241, 1242], for example, found that 5-HT has a highly significant ($P > 0.01$) antidiuretic effect in well hydrated rats in subcutaneous doses as low as $10 \mu\text{g/kg}$ and that this effect cannot be ascribed either to lowering of blood pressure or to painful sensations produced by the injection. Without excluding the possibility that 5-HT directly stimulates the kidney tubule to cause increased water reabsorption, the main cause of reduced diuresis is considered to be a diminished glomerular filtration produced by changed blood flow. The fact that a very slight drop in filtration can cause a large drop in water output is taken as a satisfactory explanation of the sometimes observed dissociation between diuresis and creatinine excretion, the latter being less reduced than the former.

The subcutaneous administration route of 5-HT is far more efficacious in producing antidiuresis than intraperitoneal or intravenous routes [9, 364]. However, a transient sharp drop in the rate of urine flow is obtained in hyperhydrated rats even by injection into the femoral vein of doses of 5-HT as low as $0.5 \mu\text{g}/100 \text{ g}$ [1122], and in rats brought into osmotic diuresis by intravenous infusion of $2 \mu\text{g/kg/min}$ 5-HT [317, 318].

The antidiuretic effect of 5-HT is significantly reduced by pretreatment with chlorpromazine (50 mg/kg) [297, 298] and much more so by pretreatment with BOL and LSD ($50\text{--}500 \mu\text{g/kg}$). The lysergic acid derivatives are ineffective when given after 5-HT [317, 318].

As already stated, the site of action of 5-HT in the rat kidney is believed to be mainly vascular. Additional strong evidence supporting this view is afforded by the vasoconstriction seen in direct perfusion experiments of the kidney [244] and, even more, by the severe acute and chronic morphological alterations found in the kidney of animals treated with high doses of 5-HT (see page 249).

In isolated rat kidneys perfused with isotonic dextran solutions the flow rate is reduced by 25 and 60% after addition to the liquid of 5-HT in concentrations of 0.034 and 0.125 $\mu\text{g/ml}$, respectively. Even maximal effects of 5-HT can be fully reversed or suppressed by suitable doses of LSD (0.2 $\mu\text{g/ml}$) [910b].

The opinion of BENDITT [87] that trapping of fluid peripherally seems to offer an adequate explanation for the antidiuretic effect of 5-HT administered by the subcutaneous route cannot be seriously taken into consideration. Similarly, all the available experimental evidence is against the participation of post-pituitary ADH in the production of the antidiuresis caused in rats and mice by single low doses of 5-HT [298, 244, 1242].

This statement is not in contrast with the histochemical findings of KIVALO *et al.* [658, 660] that repeated subcutaneous doses of 5-HT (0.2 mg/kg) may provoke a depletion of the neurosecretory substance in the hypothalamus and in the posterior lobe of the hypophysis.

Mice behave like rats in regard to the action of 5-HT on the kidney [1262].

Besides 5-HT, both 5-HTP and 4-HTP possess a conspicuous antidiuretic effect and produce cortical necrosis after having, of course, been decarboxylated to the corresponding amines [386, 386b]. The minimum doses of the racemic aminoacids active on diuresis are 10 and 20 mg/kg, respectively.

5-Hydroxy-N-acetyltryptophan and 5-acetoxy-N-acetyltryptophan also cause antidiuresis, but they are considerably less effective than 5-HTP, even though they cause the appearance in the urine of large amounts of 5-HT. This demonstrates that only 5-HT reaching the kidney via the blood is capable of reducing urine flow, whereas the amine generated within the kidney by decarboxylation of 5-HTP is inactive [385].

The isomers of 5-HT are devoid, or nearly so, of any antidiuretic activity. This is true even for 4-HT, the precursor aminoacid of which, as stated above, definitely reduces urine flow.

Concerning the antidiuretic effect of 5-HT in rats, emphasis should be laid on the fact that this effect is obtainable even by oral administration of L-tryptophan, the remote precursor of 5-HT [102]. It is exceptional to produce, in normal non-premedicated animals, acute pharmacological actions by single oral administration of a common dietary aminoacid. This suggests that in rats (only in rats, however, and perhaps in mice) 5-HT may actually participate in the physiological control of renal function.

Dog. The antidiuretic effect of 5-HT in the dog as well as in all other animal species examined is considerably less intense and constant than that observed in rats and mice. If we add that practically every research worker has used, in investigating the effect of 5-HT on the dog kidney, different doses of 5-HT, different routes of administration and different methods, it is small wonder that

results are often at apparent variance and that the mechanism of action of the drug in the dog kidney is controversial and largely obscure.

The main experimental results can be summarized as follows:

a) The minimum antidiuretic dose by single intravenous injection varies from 10 to 20 $\mu\text{g/kg}$ and by intravenous infusion from 5 to 10 $\mu\text{g/kg/min}$. The subcutaneous or intramuscular doses required to produce antidiuresis are considerably higher [2, 125, 1163]. Surprisingly enough, by the venous route 5-HT is more effectively antidiuretic when injected into the cubital vein than when injected into the malleolar vein [2].

Given by single intravenous injections (20–30 $\mu\text{g/kg}$ and more), 5-HT not only reduces urine flow in both innervated and denervated kidneys, but also reduces the renal clearance of diodone and creatinine. The kidney surface becomes pale for several minutes, and injected bromophenol is found abundantly in the medullary zone, but only in a few radial streaks in the cortex. Juxta-medullary glomeruli are devoid of dye [1008, 2].

The renal venous pressure shows only a small transient reduction [800].

It should be emphasized that the antidiuretic effect varies greatly from one animal to another and in the same animal from one observation period to another [2, 921].

b) 5-HT administered by constant intravenous infusion at a rate of 5–10 $\mu\text{g/kg/min}$ produces in trained unanaesthetized dogs a more or less marked reduction of urine flow and of sodium excretion, a significant decrease in glomerular filtration rate and an elevation of the effective renal plasma flow. Generally, antidiuresis and increase in RPF appear at dose levels lower than those required to produce a decrease in GFR. Often antidiuresis is followed by diuresis lasting 30 to 45 minutes. The above effects occur in the absence of any significant change in the mean arterial blood pressure [1162, 1163, 890, 125].

c) 5-HT infused at a rate of 1.5–3 $\mu\text{g/kg/min}$ into dogs infused with isotonic NaCl does not significantly alter urine flow, RPF or GFR but significantly decreases electrolyte excretion [745, 745b].

d) 5-HT infused directly into the renal artery of laparatomized, anaesthetized dogs at a rate of 4–40 $\mu\text{g/min}$ produces an increase of the geometric component of renal vascular resistance through some mechanism unrelated to extrinsic nerves or to endogenous catecholamines. The constriction of the renal vascular bed produced by 4 $\mu\text{g/min}$ appears to be nearly maximal. 5-HT has little local effect upon urine flow rate [356].

Whereas single injections of 5-HT were found by PAGE [890] to produce a strong and sustained constriction in perfused dog's renal vessels, the same injections appeared to possess, in the experiments of CERLETTI *et al.* [244] only a slight and temporary constrictor effect, at least 100 times less intense than that provoked by the same dose of adrenaline.

Similarly, 5-HT perfused through the kidney in concentrations up to 7 $\mu\text{g/ml}$ caused no significant change in the renal blood flow [288].

e) In a dog suffering from diabetes insipidus urine flow was reduced by 30 to 40% with 15 $\mu\text{g/kg}$ 5-HT given intravenously, and by 40–70% with 30 $\mu\text{g/kg}$

5-HT [2]. This is another piece of evidence suggesting that 5-HT antidiuresis is independent of the integrity of the posterior pituitary lobe [60, 125].

It is rather difficult to draw from the preceding data any definite conclusion about the mechanism of action of 5-HT on the dog kidney.

Whereas ABRAHAM [2] and PICKFORD [921] believe that it is necessary to look beyond the kidney for the effect of 5-HT on urine flow, suggesting that this is dependent on changes in the systemic blood pressure brought about by reflexes set up from the thorax, the great majority of investigators point to a direct action of 5-HT on the tubules and/or the intrarenal vascular bed. Concerning the possible vascular action of 5-HT, it should be remembered that both a decrease in renal vascular resistance (dilation of the afferent and efferent arterioles), and an increase in this resistance (constriction of the afferent arterioles) may produce a fall in intraglomerular pressure and, as a consequence, a fall in GFR.

Cat. In cats slightly anaesthetized with chloralose small intravenous doses of 5-HT (threshold dose $3 \mu\text{g/kg}$) reduce the flow of urine into the bladder for $1\frac{1}{2}$ –2 minutes. Paling of the kidney has never been observed, even after $50 \mu\text{g/kg}$ doses. Antidiuresis is followed by a secondary diuresis.

The mechanism of this temporary antidiuretic action is obscure. Reflex fall in blood pressure seems to play little part and FASTIER and WAAL [392] similarly hesitate to attribute the antidiuretic effect of 5-HT in the cat to either ureteral spasm or to constriction of renal blood vessels. In fact, if it is true that a moderate reduction of blood flow through the kidney was observed by CERLETTI *et al.* [244] in direct perfusion experiments it should be noted that the required doses of 5-HT were rather high (10 – $20 \mu\text{g}$) and that adrenaline appeared in this preparation, too, to be 15 – 20 times as active as 5-HT.

Rabbit. Intravenous doses of 5-HT of the order of 20 – $200 \mu\text{g/kg}$ constantly produce striking and persistent paling of the kidney [392]. Ischemia is particularly evident in the cortex. Concomitantly, there is a drop in the flow of urine from the urethers [1008].

Man. Normal subjects. As in the case of the dog, the effect of 5-HT on diuresis and renal circulation varies conspicuously not only with the dose and the manner of administration of the drug, but also with the methods used in evaluating the effects of 5-HT on the kidney. Moreover, under exactly the same experimental conditions, striking differences in the reaction to 5-HT may be observed in different individuals.

On the whole, antidiuresis is the most frequent response to 5-HT.

HOLLANDER and MICHELSON [591] found that following intravenous injection of 0.5 mg 5-HT base (approximately $10 \mu\text{g/kg}$) urine flow is reduced moderately but significantly for about 45 minutes. Concomitantly there is a slight but significant decrease in RPF often, but not necessarily, accompanied by a reduction in GFR and excretion of sodium and potassium. These investigators conclude that 5-HT acts as a moderate antidiuretic but a weak renal vasoconstrictor agent.

DEL GRECO *et al.* [318] and SCHNECKLOTH *et al.* [1068] observed that during osmotic diuresis 5-HT is marginally effective even when infused at a

rate of 2 $\mu\text{g/kg/min}$ and that doses of 4–9 $\mu\text{g/kg/min}$ reduce not only urine flow but also RPF and Na output; SINCLAIR [1122] as well as AMBANELLI and SALVI [12] have shown that when doses of 4–8 mg 5-HT are given by the intravenous route, simultaneously with a rapid intravenous infusion of 850 ml of isotonic dextrose, the duration of the diuretic response tends to be shortened; in some subjects the urine eliminated in the 90–150 minute period following 5-HT administration being barely 30–40% of that eliminated by the controls.

To the above positive results, those of other investigators should be added showing that antidiuresis produced by 2–4 mg 5-HT given by the intravenous or intramuscular route [21, 22, 272] is particularly evident after a water load, that GFR varies rather slightly, in general in the direction of a diminution [21, 22, 238, 239], that RPF varies insignificantly in some cases, but presents a clear decrease in others [238, 239], and finally that there is generally a reduction in NaCl excretion [603, 272].

At complete variance with the preceding data are those of other investigators who find that 5-HT does not produce any change in the renal function or circulation of normal subjects [269, 228, 1249, 286, 867]. However, in this regard it immediately must be stressed that some of the negative results cannot be taken into consideration because of unsuitable experimental procedure, e.g. determination of the urine excretion only at the end of a 24 hour period [228, 286, 1249], and that in other cases negative conclusions are not in accordance with partly positive experimental results [207].

Diabetes insipidus. Results obtained in this disease are discrepant: those of SERRAVALLI [1103] and of CURTI and FORNAROLI [286] being negative, while those of ANNONI and LUCHELLI [21] are positive. The latter reported that in a case of juvenile diabetes mellitus with polyuria, 5-HT (2.2 mg every 8 hours, i.m.) reduced by 70% not only diuresis but also glycosuria.

Carcinoidosis. In some cases of carcinoidosis occasional periodic oliguria is observed clinically, often preceding the flushing attacks [1122]. During study of kidney function, severe renal vasoconstriction has been found in some instances unassociated either with hypertension or with abnormalities of renal function [318, 1068].

It is difficult from the above data to draw definite conclusions on the site and mechanism of action of 5-HT in the human kidney. Evidence in favour of a direct vascular action of the amine is balanced by evidence in favour of a direct action on the tubules.

5-HT has been considered for some years by the writer [364] as a hormonal substance participating in the regulation of the intrarenal circulation and the function of the kidney. In spite of the fact that the existence of an antidiuretic effect of 5-HT generally has been confirmed in all the mammals studied, it must be admitted that so far only for rats do the renal actions of 5-HT fulfil the main conditions necessary for them to be considered 'physiological'. For all the other mammalian species, the renal effects, besides being more irregular and inconstant, are obtained with high, unphysiological doses of 5-HT.

Thus, the theory of a specific renal effect of 5-HT should be dropped or drastically limited as lacking sufficient experimental evidence.

6.8 Action on extravascular smooth muscles

6.81 Gastro-intestinal tract

Man. Single intravenous injections of 5-HT produce in normal volunteers, studied by the balloon-kymographic method, a sharp rise in intestinal tone and the disappearance of mixing waves. In 25% of the subjects doses as low as 0.4 mg 5-HT base have a pronounced effect; however in another 15% doses up to 1.5 mg are ineffective and in yet another 10–15% 5-HT produces a decrease in tone. The stimulant effect appears 30 seconds after the injection and disappears within 3–4 minutes, followed by a period of hypomotility and refractoriness to 5-HT [262, 566]. The stimulant effect of 5-HT (0.2–0.6 mg base) appears to be particularly evident in the duodenum and jejunum, less constant in the middle intestine and absent in the ileum and large intestine [104c]. The increase in tone is accentuated by prior administration of MAO inhibitors, inhibited by anticholinergic drugs and left unchanged by hexamethonium. 5-HT in doses up to 13 mg of the base fails to elicit any intestinal response when given intrajejunally to normal volunteers. However, 12 cirrhotic patients and two patients with non-tropical sprue showed a typical response when 8 mg 5-HT was instilled into the jejunum. It has been suggested that this hypersensitivity may be due to a deficit of MAO [262, 566].

In patients with ileostomies the intravenous injection of 1 mg 5-HT base produced an initial spasm of the intestine, followed by rhythmic contractions which lasted several minutes and then by a period of mechanical inactivity. The electrical activity of the ileal bud just distal to the intestinal segment containing the balloon was characterized by an initial continuous burst of action potentials followed by inhibition of electrical activity and then by intermittent bursts of action potentials. The effect of 5-HT was not inhibited by atropine, morphine and promethazine, but was blocked by large doses of LSD [296c].

The above observations have been substantially confirmed by experiments in which 5-HT has been given by intravenous infusion, at rates of 0.1–0.6 mg/min. Again it was seen that 5-HT, while stimulating the musculature of the jejunum and the ileum, also inhibited spontaneous movements, and eventually tonus of the stomach and the large intestine [1076]. Moreover, the stimulant action produced by infused 5-HT caused tachyphylaxis [558]. BOL effectively counteracts the effects of 5-HT, while atropine is inactive [558].

The effect of intravenous 5-HTP (threshold dose 100–200 μ g/kg given over a period of 5 minutes) differs from that of 5-HT in that the amino acid provides a more sustained stimulation of the small intestine [558] with inhibition of the stomach and colon and no tachyphylaxis [1082]. The stimulant effect of 5-HTP is inhibited not only by BOL (2 mg, orally) but also by atropine (1 mg, i.v.) [558].

Dog. 5-HT in intravenous doses as low as 4 $\mu\text{g/kg/min}$ of the base, is a potent stimulus to intestinal motility in the dog. Response usually starts after 4–5 minutes but tachyphylaxis develops during continuous infusion, irrespective of the dose. Within 10 minutes after cessation of the infusion the bowel regains its former sensitivity. The action of 5-HT is not blocked by atropine, hexamethonium or mepyramine. Surprisingly enough, it is potentiated by LSD [556].

Given by injection into a terminal artery of the colon 5-HT is as potent as acetylcholine in its contractile effect upon the intestinal musculature, the threshold dose varying from 0.001 to 1 μg . Atropine is 100 times more active towards acetylcholine than against 5-HT. Hexamethonium is ineffective, whereas BAS and prochlorperazine are effective antagonists [1132, 1133].

The intestinal villi are stimulated in their rhythmic automatic activity both by intra-arterial and intravenous 5-HT (4–40 μg base), as well as by direct application of the drug on the exposed mucosa (4 $\mu\text{g/ml}$). The effect lasts for about 10 minutes and is reversible. It is blocked by LSD and chlorpromazine, but not by atropine [751].

Hypermotility of the gastrointestinal tract occurring in dogs above an intestinal obstruction, and present also after hepatectomy or Eck fistula, was found by TYCE *et al.* [1221b] to be accompanied by marked increase (up to 500%) of the 5-HT content in the pyloric mucosa, and by slight increase in the duodenal mucosa. No changes were appreciable in the 5-HT content of jejunal and ileal mucosae, nor in the blood and brain 5-HT levels. Similarly, the urinary excretion of 5-HIAA was normal.

Rabbit. The rabbit intestine *in situ* is no more sensitive to 5-HT than the isolated intestine. In fact, to obtain a clear stimulant action on the intestinal tone intravenous doses as high as 0.2 mg/kg 5-HT base are required, the threshold dose being 10 $\mu\text{g/kg}$. Even at enormous dose levels (5 mg/kg, i.v.), tonus increase lasts less than one minute and tachyphylaxis appears with subsequent doses. The spasmogenic activity is abolished by atropine and Banthine®, but not by LSD [469, 470].

As in guinea-pigs, 5-HT in rabbits is also released from the intestinal wall into the lumen during peristalsis. The amount released by the caudal portion of the distal colon is barely 10% of that released by the small intestine.

At partial variance with the guinea-pig, in the rabbit 5-HT, applied either outside or inside the intestine (1–10 $\mu\text{g/ml}$), stimulates propulsive activity after an initial contraction, although the stimulant effect is less rapidly obtainable if 5-HT is applied inside [713].

Guinea-pig. Very important work has been devoted to the study of the influence of 5-HT on intestinal motility in this species and results obtained in the guinea-pig constitute the basis for the theory which considers 5-HT to be a hormone participating in the physiological regulation of peristalsis.

The influence of exogenous 5-HT and 5-HTP on peristalsis and the influence of peristalsis on liberation of endogenous intestinal 5-HT have been thoroughly investigated by BÜLBRING *et al.* and by other research workers on both isolated and *in situ* intestinal loops. The main results are as follows:

a) 5-HT (0.5–10 $\mu\text{g/ml}$) introduced into the bath in which an intestinal loop is suspended causes a transient muscle contraction followed by abolition of the peristaltic reflex [676, 186, 715, 486, 487]. This predominant inhibitory effect is constant for the small intestine, whereas as a rule the colon ascendens shows increased propulsive activity [715].

5-HT added in low concentrations (0.005–0.02 $\mu\text{g/ml}$) to the fluid outside the intestine restores slight peristaltic activity after this activity has been abolished by cooling. If the peristalsis has been depressed but not abolished by cooling, 5-HT in the bath sometimes abolishes it [76].

The transient or persistent facilitation of peristalsis produced by 5-HT applied to the serosal surface has been interpreted as being due to sensitization of the muscle to acetylcholine [186] or to facilitation of transmission at synapses involved in the peristaltic reflex arc [76]; the inhibition of peristaltic reflex has been attributed to ganglionic block [186, 715].

It is of interest that, like 5-HT, both BOL and LSD block, on external application, the emptying phase of the peristaltic reflex in the guinea-pig's lower ileum [486, 487].

b) Introduction of 5-HT into the lumen of the isolated guinea-pig intestine stimulates peristalsis in concentrations of 10^{-9} to 10^{-5} . The threshold of the intraluminal pressure required to elicit peristaltic waves is lowered, the contractions become more frequent and a larger volume of fluid is propelled [185]. LEMBECK [715], for example, found that the frequency of peristaltic waves is increased by 90 and 200% after infusion of 5-HT into the lumen at rates of 1.7 and 4.8 $\mu\text{g/min}$, respectively.

Stimulation of peristalsis is observed in isolated strips of both ileum and colon. During irregular muscular activity of the latter, 5-HT applied in the lumen abolishes non-propulsive contractions and produces co-ordinate waves; however, higher concentrations of 5-HT cause an initial contraction and stimulation of peristalsis followed by depression [713].

Mucosal application of 5-HT is highly effective in removing the block of the peristaltic reflex produced by morphine, Dibenzylamine®, procaine and 5-HT applied to the serosa, but less effective in removing the block produced by atropine and hexamethonium and completely ineffective towards that caused by BOL and LSD [186].

c) When peristalsis is recorded from loops of *in situ* intestines with normal blood supply, it is observed that in the absence of spontaneous activity at low intraluminal pressure both intraarterial (0.1–0.2 μg) and intravenous (4–10 μg) injections of 5-HT, as well as those into the lumen (100–200 μg), cause the appearance of short bursts of peristalsis. Similarly, slow intra-arterial infusion of 5-HT (0.2 to 1 $\mu\text{g/min}$) and 5-HTP (0.1 to 2.4 $\mu\text{g/min}$) causes vigorous peristalsis at pressures 10 mm below the normal threshold. However tachyphylaxis occurs readily with all three routes of administration and high doses of 5-HTP always cause a diminution of fluid transport. Desensitization is more evident *in vivo* than *in vitro* [188].

d) Small amounts of 5-HT ($0.1\text{--}0.2\ \mu\text{g}$ in 30 min) have been found to be released into the fluid flowing through the lumen of an isolated or *in situ* loop of small intestine at an intraluminal pressure subthreshold for the initiation of peristaltic waves. When the intraluminal pressure is raised and the fluid is actively pushed out by peristalsis the amount of 5-HT increases 2–8 times [185]. A further increase occurs in the presence of iproniazid, but the most striking increase (up to 2000 times the normal) is observed following intraluminal or intra-arterial administration of 5-HTP. It should be emphasized that 5-HTP produces only a small increase in the mucosal 5-HT content [188].

At a constant filling pressure the 5-HT release is much greater during bursts of peristaltic activity than during the intervening periods of rest. However, a three-fold increase of 5-HT release also may be observed in the absence of any muscle contraction when the pressure is raised [187].

The amount of 5-HT released into the lumen during peristalsis of the oral portion of the guinea-pig distal colon ($6.3\ \text{m}\mu\text{g}/\text{min}$) is of the same order of magnitude as in the small intestine ($5.7\text{--}9.6\ \text{m}\mu\text{g}/\text{min}$); whereas the amount released from the caudal portion is considerably smaller ($1.6\ \text{m}\mu\text{g}/\text{min}$). Sympathetic nerve stimulation reduces the amount of 5-HT on the average by only 30% whereas pelvic nerve stimulation causes no significant change [713].

e) Peristalsis is abnormally active in reserpine-treated animals and occurs at low thresholds. It declines gradually but does not stop in spite of extremely low mucosal 5-HT content (1–10% of the normal) and very small amounts of released 5-HT. Peristalsis does not stop in these animals even when BOL is introduced into the lumen [188].

According to BÜLBRING and LIN [185, 188b] and to LEMBECK [715] 5-HT may stimulate intestinal motility mainly by sensitizing the sensory receptors in intestinal mucosa which are sensitive to pressure and trigger the peristaltic reflex. In addition, 5-HT may also stimulate chemoreceptors whose fibres converge on the same motor ganglion cells on which the fibres from pressure receptors impinge. The stimulation of both types of receptors would be additive and the reflex would thus be triggered off more readily.

However, on the basis of results listed under (e), BÜLBRING [188] herself recently has advanced the opinion that 5-HT may not be of primary importance in initiating and maintaining peristalsis.

Two kinds of tryptamine receptors have been described in the guinea-pig ileum, namely, the M receptors which can be blocked with morphine and the D receptors which can be blocked with Dibenzylamine®. The M receptors are probably in the nervous tissue and D receptors are probably in the muscle [452, 729b].

Rat. Both 5-HT (10 mg/kg, s.c.) and 5-HTP (100 mg/kg, s.c.) first increase (by 15–20 times during the first 30 minutes) and then reduce the number and weight of faeces produced. 1 mg 5-HT or 10 mg/kg 5-HTP is just effective and 0.1 mg or 1 mg/kg is ineffective [164].

Concerning intestinal transit, as followed by the distribution of a dye or of charcoal given by mouth, both 5-HT (threshold subcutaneous dose 0.1 mg/kg; threshold oral dose 10 mg/kg) and 5-HTP (threshold subcutaneous dose 0.4

mg/kg) produce a definite inhibition of stomach emptying and intestinal transit [164, 747]. The retardation probably occurs at the pyloric sphincter since the effect disappears if charcoal is allowed to enter the duodenum before 5-HT is given [164].

Mouse. As in rats, 5-HTP produces in mice a conspicuous increase in the number and weight of faeces. The ED 50 by the intraperitoneal route is 25 $\mu\text{g}/\text{mouse}$, the ED 100 300 $\mu\text{g}/\text{mouse}$. 4-HTP gives very inconstant and variable effects, the ED 50 being not less than 400 $\mu\text{g}/\text{mouse}$ [382].

Fish. Fish afford another very impressive example of species differences in the sensitivity of the gut to 5-HT. In fact, whereas concentrations of 5-HT as low as 0.005 $\mu\text{g}/\text{ml}$ are sufficient to stimulate the isolated intestine of *Pleuronectes* and *Labrus*, concentrations 100–200 times higher are barely effective on the intestine of *Squalus*, *Raja* and *Myxine* [388].

5-HT as a hormone regulating intestinal peristalsis. Because the main sites of production and storage of 5-HT in the mammalian organism are the enterochromaffin cells of the gastro-intestinal mucosa, and because 5-HT in several species has a powerful stimulating action on intestinal motility, it has been suggested that the chief physiological function of 5-HT, or at least one of its functions, is to control motor and perhaps also secretory activity of the gut.

SMITH *et al.* [1140] and LEMBECK [715] go even further, since they believe that the effects of 5-HT outside the gastro-intestinal tract are always unphysiological, because they become apparent only after injections of exogenous 5-HT or following hyperproduction of 5-HT by malignant argentaffinomas.

The hypothesis of a local function of 5-HT within the gastro-intestinal mucosa soon after its release from the enterochromaffin cells is at first sight very attractive, and it has been recently substantiated by the important results reported in detail in the preceding pages.

On the basis of these results BÜLBRING [188b] and LEMBECK [715] maintain that the formation of 5-HT by the enterochromaffin cells could be part of the physiological mechanism required for peristalsis; more precisely, that 5-HT has a physiological modulating function in peristalsis.

The results and conclusions of BÜLBRING *et al.* are undoubtedly of great importance. However once again extreme caution seems to be necessary in generalizing results obtained in one or two animal species or in transferring experimental data obtained on isolated intestines, or even on intestinal loops *in situ*, to the gastro-intestinal tract of the intact animal. In fact, if we assume that the 5-HT which enhances the peristaltic reflex has a local origin, then it is rather difficult to give an acceptable explanation for the conspicuous differences in the distribution of the enterochromaffin cell system and of 5-HT along and outside the gastro-intestinal tract of the various vertebrate species. What could be the meaning of the enormous accumulation of enterochromaffin cells and 5-HT in the intramural portion of the pancreatic duct of the rabbit? What could be the reason for the predominant accumulation of 5-HT in the stomach of mice, hedgehogs and dogs, in the small intestines of guinea-pigs and chickens

and in the large intestines of rats, horses and cows? What, finally, is the significance of the enterochromaffin cells in the pancreatic islets of some mammals, and in the thymus of some birds? Moreover, if the 5-HT introduced into the lumen of an isolated guinea-pig intestine produces a stimulation of peristalsis in very low concentrations, the same 5-HT requires a decidedly higher dosage to produce short bursts of peristalsis when introduced into a loop of the guinea-pig intestine *in situ*, and requires a dosage far beyond any physiological limit to influence the motility of the intestine in the intact animal. In fact the threshold oral dose capable of accelerating intestinal transit in rats is 4 mg/kg of the base, and doses of 5-HT base up to 10–15 mg given intrajejunally to normal volunteers fail to elicit any intestinal or systemic response.

Finally, it has been demonstrated in recent experiments [101] that a tryptophan load which in rats is capable of doubling the rate of biosynthesis of 5-HT by the gastro-intestinal mucosa, as shown by antidiuresis and by increased 5-HIAA excretion, does not influence intestinal transit.

It is evident that the above findings and considerations are not in favour of the hypothesis that 5-HT has a *general* significance as a *local* hormone modulating peristalsis.

Of course 5-HT could also act on the gastro-intestinal tract via the blood. But, frankly, it would be rather surprising if the 5-HT released from the intestines, has to return to the same intestines, after having been exposed to severe enzymatic attacks and to large losses, to display there its main physiological action.

At any rate if there are experimental results which demonstrate that stimulation of intestinal motility may be produced in some mammalian species by physiological amounts of 5-HT injected intravenously or intra-arterially, there are other results which show that in other species parenteral 5-HT acts on peristalsis only at very high dosage levels, considerably exceeding those required to produce other biological effects, e.g., antidiuresis in rats.

The obvious conclusion is that before the hypothesis of a *general* significance of 5-HT in controlling intestinal motility can be accepted, additional positive experimental evidence, obtained in representatives of all vertebrate classes, is required, because all vertebrates contain enterochromaffin cells and 5-HT in their gastro-intestinal mucosa.

6.82 Extrahepatic biliary tract

5-HT does not display, in concentrations up to 1 mg/ml, any effect on the isolated guinea-pig biliary bladder nor, *in vivo*, on the human biliary tract, as explored radiographically (2 mg 5-HT, i.v.). The volume of the rabbit gall-bladder, on the contrary, is reduced by the i.v. administration of 0.4 mg/kg 5-HT base [492c].

6.83 Bronchial smooth muscle

5-HT given to guinea-pigs by 1% aerosol regularly produces bronchoconstriction with a shock syndrome resembling that caused by histamine or acetylcholine. However fatal shock is rare, and tolerance to this effect develops

quickly [569, 570]. Expiratory reflexes are altered and dyspnea is of the inspiratory type [817].

With aerosols containing 0.5–0.7% 5-HT initial hyperpnoea turns into definite asthmatic attacks only in 30–40% of the animals and, despite continuous administration of the aerosol, these attacks resolve spontaneously [437]. Tuberculous guinea-pigs tolerate 5-HT inhalations better than healthy animals [430].

Bronchoconstriction caused by 5-HT is antagonized by LSD (0.05–0.4 mg), adrenaline and noradrenaline. Section of the vagi and atropine, sympatholytics, pantocaine and antihistamines yield inconstant or no effect [569, 570, 817].

The isolated guinea-pig lung behaves like the *in situ* lung. Here again the bronchoconstrictor action of 5-HT (10–40 μ g) is strongly antagonized by UML, LSD (0.25–4 μ g) [1118b], adrenaline and isopropyl noradrenaline (5 μ g), and moderately so by dihydroergotamine, Dibenamine® and Regitine®.

Atropine, even at 0.5 mg doses, is a feeble antagonist and antihistamines shows no action whatsoever [54, 111]. Chlorpromazine is effective when given prior to the administration of 5-HT, but only weakly active in reversing 5-HT bronchoconstriction [651].

Surprisingly enough, 5-HT causes in all species examined only relaxation of the medium size bronchioles. The most sensitive bronchioles are those of rats and cats (0.01 μ g 5-HT/ml), followed by those of dogs (0.05 μ g/ml) and guinea-pigs (0.4 μ g/ml). The bronchioles of rabbits, monkeys and man are practically insensitive [165].

As with mammalian bronchioles, the suspended frog lung is relaxed by 5-HT, the threshold concentration being as low as 10^{-13} – 10^{-14} . In this preparation 5-HT is about 1000 times more potent than catecholamines [161].

6.84 Uterus

Man. 5-HT seems to be poorly active both on the isolated and the *in situ* human uterus. Concentrations of 5-HT as high as 10 μ g/ml are needed to cause an increase in amplitude of contractions of excised human myometrium, and intravenous infusion of 5-HT shows no definite and constant effect on the *in situ* uterus in pregnancy or labour [463].

The use of 5-HT as an oxytocic agent is therefore highly questionable [1240].

Dog. To an intravenous injection of as little as 1 μ g/kg 5-HT the uterus of both conscious animals and of animals anaesthetized with chloralose responds with a contraction, followed by a period of inhibition lasting up to 7 minutes. The action appears to be wholly peripheral [4].

Rat. No gross morphological alterations are visible in the uteri of non-pregnant rats given, over a 120 minute period, 14–21 mg/kg 5-HT base [232]. Similar doses of 5-HT (4–16 mg/kg) given to pregnant rats at various stages of pregnancy fail to interrupt pregnancy. Interruption of pregnancy, on the other hand, is observed regularly following higher doses of the amine (32–40 mg/kg given in divided doses over a period of 2 hours). Foetal death and abortion are caused by severe placental haemorrhage [231, 790b].

Mouse. When administered subcutaneously at high dose levels (1 mg/mouse/day divided into two injections) 5-HT does interrupt pregnancy both in its early and in its later stages. Only a limited number of mice become pregnant, and pregnancy is discontinued in all mice given 5-HT during the 11th to 16th day of pregnancy. At this time a single subcutaneous dose of 1 mg 5-HT is sufficient to produce death of the foetuses within half an hour. At autopsy death of foetuses is found to be caused by marked haemorrhage and disorganization of the placenta [954, 955].

Pretreatment (30 min) of the females with D-lysergic acid derivatives preserves the life of the young, the ED 50 for LSD being of 30 $\mu\text{g/kg}$ and that for UML of only 3.7 $\mu\text{g/kg}$ [428b].

Lower doses of 5-HT (0.05–0.25 mg, s.c. or i.v.) have no effect whatever on pregnancy [954, 955, 1051].

Rabbit. A daily subcutaneous dose of 2–2.5 mg/kg 5-HT causes foetal death in rabbits, as in mice and rats, when given in late pregnancy (25th to 30th day), but has no effect on implantation and early foetal development. In late pregnancy, foetal death also occurs when 5-HT is administered by intravenous infusion (10 mg over 45 min), and large haemorrhagic areas become visible in the placenta [954, 955].

It seems probable that the interruption of pregnancy seen in rats, mice and rabbits following large doses of 5-HT is due to a direct action on the placental vessels which are known to be particularly sensitive to the drug.

Owing to the enormous, extraphysiological doses of 5-HT required to produce placental haemorrhage and disorganization in experimental animals, any connection of these phenomena with human placental apoplexy or toxæmia of pregnancy is improbable.

6.85 Ureter

The perfused *in situ* dog ureter, with blood supply intact, responds to intravenous 5-HT (threshold dose 3–4 $\mu\text{g/kg}$) with a contraction which completely prevents the passage of fluid through its lumen for 1 to 2 minutes and slows the flow for a further period of 1 to 2 minutes [3].

The isolated dog ureter, on the other hand, does not react to 5-HT, even at concentrations up to 4–20 $\mu\text{g/ml}$ [1164, 148].

6.86 Nictitating membrane

5-HT and related indolealkylamines contract both the *in situ* and the isolated membrane, and antagonize or potentiate, depending upon the dose, the response to adrenaline [275, 279, 706, 1086], and to pre- or postganglionic stimulation of adrenergic nerves [45b].

The threshold intravenous dose causing the normal and denervated membrane to contract is 14.4 and 1.69 $\mu\text{g/kg}$ for 5-HT, and 30.8 and 4.27 $\mu\text{g/kg}$ for bufotenine [275, 279]. However, large amounts of 5-HT (100–300 μg) cause a greater contraction of the normal membrane.

Imipramine (0.15–2.4 mg/kg) potentiates by 3 to 7 times the spasmogenic action of 5-HT [527c, 1146b].

In the perfused head of the cat 5-HT has an adrenaline-like action on the nictitating membrane but is less effective than adrenaline. In this preparation 5-HT in all doses causes a larger response on the denervated than on the normal side. The direct action of 5-HT is not blocked by nicotine, but it is potentiated by cocaine [1208].

On the innervated membrane 5-HT is 5 times as potent as tryptamine [706], and 2–5 times more potent than 4-HT [382]. Single intravenous doses of 2–5 mg/kg 5-HTP do not contract the membrane; the same doses of 4-HTP produce a contraction lasting 30–50 minutes [382].

The isolated membrane brought into contraction by 5-HT (threshold dose 0.05–0.1 $\mu\text{g/ml}$) relaxes only slowly after washing out the drug and tachyphylaxis appears following successive administration of the same amount of 5-HT. It is not possible to obtain maximum contraction of the muscle even with large doses of 5-HT. Contractions are potentiated by cocaine and antagonized by atropine, sympatholytic drugs and LSD [1197].

6.87 Chick amnion

This preparation is of interest because it is devoid of nervous elements of any description. Generally 5-HT increases motility in a rhythmic preparation, already at concentrations as low as 0.01 $\mu\text{g/ml}$; quiescent strips require higher concentrations. Low or moderate doses of 5-HT give response which are reproducible without any sign of tachyphylaxis [406].

6.88 Molluscan smooth muscle

5-HT solutions in concentrations as low as 0.001–0.01 $\mu\text{g/ml}$ display a powerful relaxing action on the anterior bissus retractor muscle of *Mytilus edulis* when this muscle is contracted by acetylcholine, electric stimulation, or electric stimulation plus adrenaline [1218, 1219, 1220, 213]. In the presence of 5-HT, tension development by electrical stimulation or acetylcholine is not blocked, but potentiated, while the prolonged phase of contraction is abolished. Moreover, in the presence of 5-HT acetylcholine evokes a succession of spike-like electrical discharges [607b, 1221]. 5-HT itself, up to 10 $\mu\text{g/ml}$, does not interfere with neural excitation [1220, 1221]. LSD is ineffective as an antagonist of 5-HT on this muscle [214].

It has been suggested that the relaxing effect of 5-HT involves an alteration of membrane properties and is linked with an increased tendency of the muscle to spike and exhibit rhythmicity on stimulation [1221].

Like the *Mytilus* muscle, the muscles of the radula apparatus of *Busycon* [579] and *Buccinum* [389c] are relaxed by 5-HT and brought into rhythmical activity in the combined presence of 5-HT and acetylcholine.

TWAROG [1220] maintains that acetylcholine and 5-HT should be considered as mediators released by the nerve supplying the bissal retractor muscle,

with 5-HT being the transmitter involved in neurally-induced relaxation. This opinion, which is shared by HOLGATE and CAMBRIDGE [590] and by LOWY and MILLMAN [750], is strongly supported by the presence in the muscle of 5-HT and acetylcholine, as well as of cholinesterase and monoamine oxidase.

6.89 Muscles of the liver fluke (*Fasciola hepatica*)

5-HT and related indolealkylamines, as well as LSD have a stimulatory effect on the rate and amplitude of contractions and on the tone of the musculature of the liver fluke. The effect is peripheral and is not mediated through the central ganglion. LSD is about 20 times as active as 5-HT (threshold concentration 10 $\mu\text{g/ml}$) and this in its turn is 5–10 times more active than tryptamine or bufotenine and 100 times more active than 5-HTP.

BOL, yohimbine, harmine and dopamine depress rhythmical movements and antagonize the stimulant action of 5-HT and LSD.

On the basis of the above effects and on the occurrence of 5-HT in the organism of *Fasciola hepatica*, MANSOUR [773, 774] suggests that 5-HT or a related compound may be the humoral transmitter for the peripheral receptors of this worm.

6.810 Muscles of sea anemones

Anemone preparations (*Metridium senile* and *Calliactis parasitica*) have been found to be poorly sensitive to indolealkylamines, but more so to tryptamine (100 $\mu\text{g/ml}$) than to 5-HT [1001].

It has been suggested recently by WOOLEY and CAMPBELL [1327, 1331] that 5-HT causes contraction of smooth muscle by carrying Ca^{++} ions across the membrane into the muscle cell. This interesting hypothesis awaits the support of additional evidence.

6.9 Action on secretions

6.91 Gastric secretion

Man. At variance with CALÌ and CORDOVA who have found that intravenous 5-HT (2 mg) produces in man a stimulant action on gastric secretion [209, 206], which is inhibited by antihistaminic drugs [209], all other research workers [559, 558, 993, 1076, 1082, 920] have observed that 5-HTP and 5-HT inhibit volume and acidity of both spontaneous and histamine-induced gastric secretion, while at the same time increasing production of mucous [993, 920].

Following the intravenous infusion of 1.5–15 $\mu\text{g/kg/min}$ 5-HT maximum effects appear after 5–25 minutes and the secretory response wears off within 15–20 minutes after the infusion is discontinued [1076]. 5-HTP infused intravenously for 10 minutes at a rate of 10–80 $\mu\text{g/kg/min}$ produces a more intense and sustained reduction of gastric secretion [1082].

Dog. BLACK *et al.* [121, 122, 1138] have observed that in anaesthetized dogs starved for at least 12–24 hours and prepared with a stomach fistula and a ligature at the pylorus-duodenal junction, intravenous infusion of 5-HT at a

rate of 5–20 $\mu\text{g/kg/min}$ for 45–90 minutes does not evoke acid gastric secretion but appears to increase the production of alkaline mucous. When an acid gastric secretion is stimulated by a continuous infusion of histamine, concurrent infusion of 5-HT for 30 minutes inhibits the secretion. Recovery from the inhibition usually begins within 60 minutes after stopping the 5-HT infusion. In dogs starved for 36 hours it has been found that 5-HT fails to inhibit histamine-stimulated secretion, whereas when food is allowed during the 12-hour pre-operative period, the inhibitory effect of 5-HT is enhanced. Infusion of 5-HT at the start of histamine-stimulated gastric secretion fails to prevent the onset of secretion due to infusion of histamine.

The fact that 5-HT given after bilateral vagotomy often fails to inhibit histamine-stimulated secretion suggests the possibility that 5-HT may exert its effects on gastric secretion reflexly.

The above observations have been confirmed by HAMMOND [541] and by WHITE and MAGEE [1310], who demonstrated that increased mucin secretion is reduced by atropine but not by hexamethonium.

Results are partly at variance concerning the effects of 5-HTP. In fact, whereas BLACK *et al.* [1139, 123] found that i.v. infusion of doses of 5-HTP as low as 5–20 $\mu\text{g/kg/min}$ produces, after a latent period of 15–30 minutes, a marked fall in secretory output elicited by histamine, HAVERBACK *et al.* [557] failed to observe any inhibition of the secretory response to histamine with single i.v. doses of 5-HTP as high as 20–25 mg/kg. Yet, at this dosage level the aminoacid inhibits not only spontaneous gastric secretion (from 20 ml and 93 m Eq. per litre / 40 min to 2 ml and 0 m Eq. per litre / 40 min) but also secretions elicited by insulin and urecholine.

Cat. The effect of intravenous 5-HT (1–6 mg base/cat) on the secretion of gastric pouches is variable, in part depending upon the integrity of the vagus nerves. With intact vagi, spontaneous secretion is but little affected by 5-HT (production of a small amount of non-acid juice) and histamine-induced secretion is generally reduced in both volume and acidity. With cut vagus nerves, non-acid spontaneous secretion is more abundant, and reduction of histamine-induced secretion is sometimes preceded by an increase of secretion [308].

Guinea-pig. 5-HT given in intraperitoneal doses of 1–20 mg/kg reduces the concentration of free acid and raises the concentration of Na in the gastric secretion induced by histamine. Small doses of 5-HT increase, and larger doses decrease the peptic activity of this juice [1317b].

Rat. Both 5-HT (4–8 mg/kg, s.c.) [1098] and 5-HTP (10 mg/kg) [1099] produce a significant depression in volume, acid and pepsin output of the interdigestive gastric secretion in the rat, as well as a depression of gastric secretion stimulated by urecholine [560].

5-HT base does not produce, at intraperitoneal doses of 6 mg/kg, any significant increase in plasma pepsinogen, i.e. it does not stimulate gastric endocrine function [677b].

The suggestion by some investigators that 5-HT may play a role in the physiological regulation of the secretion of gastric juice is not supported by

adequate experimental evidence. In fact, the doses of exogenous 5-HT required to influence both spontaneous and histamine-induced gastric secretion are always unphysiological, and, on the other hand, there is no proof that endogenous 5-HT contained in the gastric mucosa may act as a local hormone stimulating gastric secretion.

6.92 Pancreatic and biliary secretion

Rats given daily injections for 3 days of 2 mg/kg 5-HT base display a slight decrease in the lipase and protease activity of the pancreatic juice, whereas the amylase activity remains unchanged [828].

In dogs with total pancreatic fistulas, 5-HT (0.1 mg base/kg, i.v.) markedly reduces both food-stimulated and non-stimulated flow of pancreatic juice. 5-HTP (25 mg/kg, i.v.) produces similar but slower and less pronounced effects. Secretin-stimulated pancreatic juice flow rate is not affected by 5-HT and 5-HTP. These drugs decrease pancreatic juice bicarbonate levels but do not affect pH or amylase activity [338b].

In man intravenous infusion of 4 μ g/kg/min 5-HT for 60 minutes has no significant effect on external pancreatic secretion (volume, bicarbonate concentration and amylase concentration of the duodenal aspirate), but partially inhibits the bile pigment flow [920].

6.93 Submaxillary secretion

Single injections of from 50 to 100 μ g 5-HT into the common carotid artery of the dog produce a potentiation of salivation caused by continuous electrical stimulation of the chorda tympani. 5-HT in this dose range produces no salivation by itself. The intravenous administration of iproniazid or reserpine further increases the potentiation observed with identical doses of 5-HT. On the other hand, it seems that 5-HT by subminimal infusion potentiates the sialagogue effect of acetylcholine.

PETRUCELLI *et al.* [916c] suggest that 5-HT, while not strictly necessary for neural transmission, may facilitate transmission at parasympathetic ganglia and/or neuroeffectors.

6.94 Sweat secretion

In man intramuscular doses of 2 mg 5-HT are sufficient to depress sweat secretion. In 50% of the cases inhibition is preceded by stimulation [849]. In mice a complete inhibition of plantar transpiration is observed only with doses of 5-HT base as high as 5–6 mg/kg [135], which are known to produce conspicuous vascular effects.

6.10 Action on respiration

The respiratory effects of 5-HT, already carefully studied by the earlier investigators have been further investigated in recent years without, however, any definite conclusions in regard to the site and mechanism of action of 5-HT.

Man. 0.5–1.5 mg 5-HT given by rapid intravenous injection produces in non-asthmatic subjects a short-lasting (20–40 seconds) hyperpnea and tachypnea, without signs of bronchial obstruction. The respiratory response is potentiated by pyrilamine, inhibited by BAS and left unchanged by anticholinergic drugs, and by ganglionic or adrenergic blockade [591, 827].

The effect of 5-HT given by intravenous infusion in doses of 1–3 mg/minute is essentially the same. Stimulation of respiration is most marked in the first 5 minutes of infusion, then diminishes in intensity without ceasing. Tidal air always increases but respiratory rate shows a variable change. On repeated infusion tachyphylaxis of the hyperpnoea is observed [900].

In asthmatic patients aerosolized 5-HT produces a prompt fall in vital capacity and a decrease in respiratory flow rate [827].

Dog and cat. It has been confirmed that intravenous injection of 5-HT in dogs causes initial respiratory stimulation followed by apnoea, whereas in cats apnoea is the first and predominant response to the drug [681]. Tidal air is reduced both in anaesthetized and spinal cats (threshold dose 1.2–15 $\mu\text{g/kg}$, 5-HT base, i.v.), probably owing to bronchoconstriction [671].

As a consequence of its respiratory stimulation, 5-HT produces in the intact dog a rise in arterial oxygen saturation, as measured by the cuvette oximeter [862].

Guinea-pig. Intravenous 5-HT constantly provokes hyperpnoea over a wide dosage range [1305]. Tidal air, however, is decreased at constant stroke volume, probably as a consequence of bronchoconstriction. The effect of 5-HT on tidal air is antagonized by LSD, BOL and 1-acetyl-LSD, and, to a considerably lesser extent, by atropine and antihistamines [671].

Rat. The respiratory response of rats to intravenous 5-HT shows remarkable individual variations [1305]. Generally brief initial stimulation is followed by apnoea in the expiratory position and then, occasionally, by moderate tachypnoea. The respiratory stimulant effect of 5-HT (4–10 $\mu\text{g/kg}$) is abolished by inactivation of the carotid sinuses but not by vagus section or ganglionic blockade [1033].

6.11 Action on enzymes. Metabolic effects

6.111 Action on carbohydrate metabolism

Mammals. In confirmation of previous results, it has been shown that both 5-HT and 5-HTP produce in rabbits a rise in blood sugar level, the maximum being attained after two hours.

Increases are by 12, 39 and 60% after 2, 4 and 13 mg/kg 5-HT base, respectively [801, 802], and by 40% after 5–20 mg/kg 5-HTP [673]. Dihydroergotamine antagonizes 5-HT hyperglycaemia, whereas pretreatment with 5-HT (1 mg/kg) has little influence on hyperglycaemia produced by LSD [672].

In human subjects 4 mg 5-HT, given i.m. or i.v., produces no appreciable change in blood sugar level [1056].

In normal dogs intravenous doses of 5-HT base as low as 5 $\mu\text{g/kg}$ fail, as expected, to produce hyperglycaemia. However, when the same or even half the amount of 5-HT is injected into depancreatized dogs, there is a significant rise in blood-sugar level during the first 40–60 minutes. This preliminary observation of SIREK [1121] still awaits confirmation.

High doses of 5-HT (2 mg/kg of the base, i.v.) produce in normal dogs a 30% rise in blood glucose, a decrease in liver glycogen and an increase in liver phosphorylase activity. All phenomena are probably secondary to a release of adrenaline from the adrenal glands [264b].

In rats, 5-HT (0.4 mg 5-HT base/rat, i.v.) induces initial hypoglycaemia followed by marked hyperglycaemia, enhanced insulin-induced hypoglycaemia and reduced adrenaline-induced hyperglycaemia. Removal of adrenal medulla greatly inhibits the hyperglycaemic response. Furthermore, the same dose of 5-HT depletes liver glycogen, decreases glycogen levels (by 35–50%) and phosphorylase activity of uterine smooth muscle, decreases glycogen concentration in the diaphragm (25–30%) and rapidly elevates glycogen in heart muscle (30%), while leaving phosphorylase activity unaltered in both instances [717, 663b].

Molluscs. When an excised gill of *Mytilus edulis* is incubated in sea water, both the rate of endogenous respiration and the rate of acid production under anaerobic conditions are stimulated promptly by the addition of 5-HT. A similar stimulation has been observed to develop slowly in the presence of 5-HTP. Concomitantly the breakdown of tissue glycogen is accelerated. In contrast neither acetylcholine nor adrenaline influences the respiration of excised gill. MILTON and GOSSELIN [833b] surmise that these metabolic effects of 5-HT are related to the cilio-acceleratory action of 5-HT observed on the gill plates.

Liver fluke. It has been reported elsewhere that 5-HT stimulates the rhythmical movement of this parasite. This stimulation is accompanied by the following biochemical effects when 5-HT is put into contact with the liver fluke at concentrations of 10^{-5} to 10^{-3} M:

- a) an increase in glucose uptake,
- b) an increase in glycogenolysis when the organisms are cultured in media containing no glucose,
- c) a 2- to 10-fold increase in lactic acid production with little or no change in the production of propionic and acetic acid,
- d) an increase in phosphorylase activity, with a rapid and specific increase in the formation of adenosine – 3', 5' – phosphate [779, 778, 780, 782, 781],
- e) an increase in the activity of phosphofructokinase, the activating effect of 5-HT being dependent upon the presence of a particulate fraction, ATP and Mg^{++} [782b].

It has been suggested that 5-HT may have a direct effect on mechanisms concerned with the uptake or utilization of glucose by flukes [779, 778, 780], and that some actions of 5-HT in the liver fluke appear analogous to some actions of catecholamines and glucagon in mammalian liver and to some actions of catecholamines in mammalian skeletal and cardiac muscle [781].

6.112 Action on oxygen consumption

0.4–4 mg/kg 5-HT base, given by the subcutaneous route, produces in rats a significant and prolonged decrease in O_2 consumption and a fall in body temperature when the environmental temperature is 20°, but has no effect when the animals are kept at 30° C [429]. Adrenalectomy does not appreciably influence the action of 5-HT on oxygen consumption while iproniazid pretreatment enhances it both in normal and adrenalectomized rats [431].

It has been suggested that reduction of oxygen consumption is secondary to the vascular effects of 5-HT.

Oxygen consumption of slides of various rat tissues incubated in Ringer-Krebs solution is moderately and inconstantly increased by 5-HT, when added to the nutrient liquid in concentrations up to 200 μ g base/ml, or injected into the animals (4 mg/kg, i.v.) 15 minutes prior to their sacrifice [826].

6.113 Action on fat content in peripheral blood

5-HT given subcutaneously to dogs in doses of 0.1 mg/kg increases neutral fats by 20% after 15 minutes and decreases phospholipids by 25% over a period of 3 hours. Free cholesterol is slightly above initial levels [790].

6.114 Inhibition of lipid peroxide formation in tissues

Because of its antioxidant activity, 5-HT has proved to be a very powerful inhibitor of lipid peroxide formation in tissues. 6–10 μ g/ml 5-HT completely blocks the lipid peroxide formation by rat liver mitochondria, and 1 μ g/ml still shows appreciable activity. 5-hydroxyindoleacetaldehyde has approximately the same activity as 5-HT; tryptamine and bufotenine are less active.

According to BERNHEIM *et al.* [92] it is not possible to state that the antioxidant activity of 5-HT explains any of its physiological actions.

6.115 Action on cholinesterase

The hydrolysis of acetylcholine, butyrylcholine, procaine and murexine by plasma cholinesterase is inhibited by high concentrations of 5-HT (10^{-3} M) and related indolealkylamines [360, 361, 362], but markedly potentiated by lower concentrations of 5-HT (5×10^{-6} M) [444]. Psilocybin and bufotenine inhibit human plasma cholinesterase more than 5-HT [1354b]. An inhibitory effect is also seen hydrolysis of acetylcholine by red cell cholinesterase; tryptamine and psilocybin is approximately as active as 5-HT, bufotenine and gramine 20 times more active [360, 1354b].

Surprisingly enough the hydrolysis of benzoylcholine by plasma cholinesterase is conspicuously activated by tryptamine, whereas 5-HT, 5-HTP and N,N-dimethyltryptamine possess no marked activating effect [362, 361].

The action of indolealkylamines on brain cholinesterase will be discussed on page 284.

6.116 Action on ceruloplasmin

5-HT, 5-HTP, 5-HIAA and bufotenine (0.38 μ M/ml), but not tryptamine and IAA, are capable of inhibiting the oxidation of N,N-dimethylparaphenylendiamine by purified plasmin and serum oxidase [25].

6.117 Action on phenol oxidases

5-HT and, to a lesser degree, other indolealkylamines, inhibit the phenol oxidases of *Fasciola hepatica* and of *Tenebrio molitor* (competitive inhibition) as well as the melanoma phenol oxidase (non-competitive inhibition), but display no effect on mushroom and potato phenol oxidase. For total inhibition of liver fluke phenol oxidase 2 mg/ml 5-HT are required, but partial inhibition is already appreciable with 0.02 mg/ml [776, 777, 1088b].

6.118 Action on tryptophan pyrrolase

Many tryptophan analogs effectively block the conversion of tryptophan to kynurenine in the tryptophan pyrrolase reaction. Among the most effective are 5-HT, 5-HTP and tryptamine, which produce a 81, 79 and 48% inhibition, respectively, at a 5×10^{-4} M concentration [444b].

6.119 Influence on the degradation of thyroxine

5-HT, 5-HTP, 5-HIAA, 3-hydroxyanthranilic acid, xanturenic acid and reserpine (2×10^{-3} M) all depress the degradation of thyroxine to inorganic iodide by homogenates of mammalian and amphibian tissues. Both 5-HT and reserpine also inhibit the formation of iodide from triiodothyronine, whereas the deiodination of mono- and diiodotyrosine by mouse liver homogenate is not altered by the presence of 5-HT [455b].

6.12 Action on chromatophores

a) *Action of 5-HT. Frog skin chromatophores.* Both in *Rana esculenta* and in *Rana pipiens* 5-HT (0.1–1 mg) injected into the dorsal lymphatic sac produces a dispersion of the pigment in the melanocytes, i.e. a darkening of the skin. According to KAHR [626, 627] the phenomenon is mediated through release of the melanocyte-stimulating hormone, but DAVEY [300] has observed, following administration of 5-HT and tryptamine, darkening of the skin even in hypophysectomized specimens of *Rana pipiens*.

VAN DE VEERDONK [1243] recently has confirmed the melanocyte-stimulating effect of 5-HT on the *in vitro* skin of *Xenopus laevis*.

Fish chromatophores. The effect of 5-HT on fish chromatophores is extremely variable depending upon the species. Whereas the drug is completely inactive, both *in vivo* and *in vitro*, on the melanophores of *Lebistes reticulatus* [242, 108], it is highly active on the chromatophores of other species. TURNER [1215], for

example, has observed that 2–3 μg of 5-HT base injected just beneath the scales of another fish produces a local constriction lasting half an hour, and that on the dilated chromatophores of the tail fin, which had been cut off, a 5-HT solution of 0.5 $\mu\text{g}/\text{ml}$ leads to contraction beginning within 10 minutes.

Although per se inactive, 5-HT is capable, given in very high doses, of antagonizing the chromatophore-expanding action of LSD and BOL, as seen in *Lebistes reticulatus* [108, 242].

Chromatophores of cephalopoda. Both the chromatophores of *Loligo pralii* [997] and those of *Octopus vulgaris* [628] are closed by 5-HT and tryptamine, the threshold concentration for 5-HT and Octopus chromatophores being 0.01 $\mu\text{g}/\text{ml}$. The same effect is produced by LSD and by monoamineoxidase inhibitors. It has therefore been suggested that chromatophore control is achieved in cephalopoda by an amine-amine oxidase system (closure) opposed by an acetylcholine-cholinesterase system (expansion) [997].

b) *Action of melatonin.* Melatonin, 5-methoxy-N-acetyltryptamine, while apparently devoid of any other important pharmacological activity, possesses a tremendously intense effect of lightening or paling on frog, toad and fish melanocytes.

The most sensitive preparation seems to be the skin of *Rana pipiens*, previously darkened with caffeine or MSH [718, 722b]. For measurable lightening of this skin the following concentrations of melatonin and related amines are required, in μg per ml: melatonin 1×10^{-7} , tryptamine 2×10^{-1} , N-acetyltryptamine 5×10^{-2} , 5-HT 1, 5-hydroxy-N-acetyltryptamine 6×10^{-3} , 5-methoxytryptamine 2×10^{-6} , 5-ethoxytryptamine 5×10^{-2} , 5-ethoxy-N-acetyltryptamine 10^{-5} , 5-methoxy-N-methyltryptamine 2×10^{-1} , 5-methoxy-N-formyltryptamine 1×10^{-4} , 5-methoxy-N-propyltryptamine 1×10^{-7} , 5-methoxy-N-butyryltryptamine 1×10^{-7} [722b, 215].

The enormous interest in the discovery of melatonin is obvious, and future studies will solve the fascinating problem of whether this hormone is involved in the function of chromatophores in human skin and in the pathogenesis of vitiligo [719, 722b].

6.13 Action on the peripheral nervous system

6.131 Excitation of afferent and efferent autonomic fibres

The following effects of 5-HT on autonomic fibres or nerve endings have been described:

- a) production of a large burst of impulses in certain vagal afferent fibres of the cat lung, the receptors of which are sensitized specifically by pulmonary deflation (6 $\mu\text{g}/\text{kg}$ into the right atrium) [891];
- b) production of a discharge of impulses in non-medullated afferent fibres in the cervical vagus and aortic nerves of the cat, coming from the gastro-intestinal tract and from the thorax, respectively (4–40 $\mu\text{g}/\text{kg}$ 5-HT base, i.v.) [337];
- c) stimulation of bronchiolar constrictory fibres which are supposed to be present in the cervical sympathetic structures of a number of dogs [189].

On the basis of the above data it has been suggested that 5-HT may have a role in the initiation of sensory signals from the lung and the gastro-intestinal tract leading by reflex to stimulation of the smooth muscles in these organs.

6.132 Action on the superior cervical ganglion of the cat

a) Intra-arterial injection of 5-HT potentiates, like pilocarpine and histamine, the response of the ganglion to submaximum preganglionic stimulation and to injections of acetylcholine, nicotine and other drugs. Response to supermaximum stimulation is not increased. The sensitivity of the perfused ganglion to the potentiating action of histamine and 5-HT is 10 to 100 times less than that of the ganglion with its normal circulation intact (threshold doses 0.1 and 0.01 μg , respectively). The potentiation of ganglionic transmission is reduced by cocaine, but remains unchanged by atropine and mepyramine.

During continuous preganglionic stimulation intra-arterial 5-HT causes an increase in the height of submaximum post-ganglionic action potentials of the S2 fibres originating in the superior cervical ganglion [1207, 1208, 1209, 1210].

b) Intra-arterial injection of 1–100 μg 5-HT causes stimulation of the ganglion in 70% of the preparations. This stimulation is abolished by nicotine and by cocaine but is not affected by hexamethonium, atropine or mepyramine [1208]. It has been suggested that 5-HT acts on sympathetic ganglia by combining with specific tryptamine receptors different from the acetylcholine receptors but similar to the 5-HT receptors postulated for nervous structures in the guinea-pig ileum [1210, 1211].

c) GERTNER [474, 473] found that harmine, iproniazid and β -phenylisopropylhydrazine produce a gradual block of transmission through the perfused cervical ganglion.

GOLDBERG and DA COSTA [504c] confirmed this observation, but showed that MAO inhibitors act only on transmission through sympathetic ganglia, without depressing the function of parasympathetic ganglia.

Even accepting the view that production of ganglionic block might be related to the ability of the above drugs to inhibit MAO, any interference of endogenous 5-HT in the phenomenon is highly questionable because the occurrence of 5-HT in mammalian ganglia has not yet been persuasively proved.

6.133 Action on the inferior mesenteric ganglion of the cat

5-HT in doses from 2.5 to 15 μg given by close intraarterial injection produces an increase in the spontaneous electrical activity of the postganglionic fibres of the inferior mesenteric ganglion. This effect is blocked by BOL, cocaine, morphine, LSD and atropine [118c].

6.134 Action on sympathetic ganglia of the rat

5-HT in concentrations as low as 1.3×10^{-6} reduces *in vitro* (isolated ganglia maintained in nerve chambers) the threshold of postganglionic responses to preganglionic stimuli and increases the amplitude of postganglionic res-

ponses evoked by submaximal preganglionic stimulation, but has no effect on direct nerve fiber transmission. This finding, supported by *in vivo* experiments, suggests, according to HERTZLER [567b], that 5-HT facilitates synaptic transmission in sympathetic ganglia of the rat.

6.135 Action on sensitive somatic fibres

It has been confirmed that 5-HT applied to the exposed base of a blister raised by cantharidin on the human forearm produces pain. 5-HT is 50 times as active as tryptamine, 4-HT 10 times as active and 6-HT twice as active, whereas bufotenine has the same potency as tryptamine. The threshold concentration for 5-HT is 10^{-6} to 10^{-7} M [31].

Injected into the safenous artery of the cat, 5-HT (1–40 μ g) causes, like histamine, at first excitation (2–4 minutes) then depression (10–60 minutes) of the slowly-adapting mechanoreceptors, with myelinated afferent fibres. Hair receptors with myelinated fibres are not altered and none or very small responses have been recorded for mechanoreceptors, heat receptors and cold receptors with non-myelinated fibres [412]. In contrast to these prevalently negative responses, 5-HT elicits, when injected arterially into the skin of the cat leg, a slowly developing discharge of impulses in non-myelinated fibres in the saphenus nerves which are commonly held to transmit painful and itching sensations. This discharge, which takes a few seconds to reach the maximum, is accompanied by more transient discharges in myelinated fibres [338].

Finally, instilled into the conjunctival sac of the rabbit eye, 5-HT potentiates, in doses as low as 2.5 μ g, the pain-producing effect of acetylcholine [799], while counteracting, at higher dosage levels (8–120 μ g), the anaesthetic action of dibucaine [1003].

However, it is highly probable that this last effect is simply due to the accelerated elimination of the anaesthetic as a consequence of the vasodilation produced by 5-HT [702, 703b].

It has been suggested by KEELE [639] that 5-HT released from blood platelets contributes, together with histamine and bradykinin-like polypeptides, to the pain which follows injury to tissues.

6.136 Action on mixed somatic nerves

5-HT does not display after local application (40–200 μ g/ml) any direct action on the excitability and conduction of the sciatic nerves of rats and frogs. The same negative result is obtained when 5-HT is given in single parenteral doses of up to 10 mg/kg. However, when the drug is administered for 3–4 days in daily doses of 4–8 mg, the conduction velocity and the height of action potentials in the nerves is significantly increased above the control values. This effect disappears following adrenalectomy and is re-established by administration of cortical extracts, suggesting that the action of 5-HT on the sciatic nerve is mediated through adrenocortical hormones [216, 217, 218, 220].

5-HT (20–200 μ g/ml) does not affect in any way the action of local anaesthetics applied on the isolated sciatic nerve of guinea-pigs [703].

Action on the neuro-muscular junction. Like catecholamines, 5-HT (25–400 µg/kg, i.v.) is capable of moderately antagonizing, both in the cat and in the rabbit, the neuromuscular block produced by tubocurarine, but not that produced by decamethonium [918, 1028].

6.14 Action on endocrine glands. Inter-endocrine correlations

Anterior hypophysis. A possible role of 5-HT in the control of ACTH liberation has been suggested repeatedly, mainly on the basis of the observation that the amine decreases the ascorbic acid content in the rat adrenal glands, and that this decrease is not found in hypophysectomized rats [850, 851], in rats whose hypothalamo-hypophysial connections have been interrupted and, finally, in animals with hypothalamus blocked by previous administration of prednisolone [1143, 1144].

However, these positive results must be completed by partially divergent findings, suggesting that 5-HT may also produce an inhibition, instead of an activation, of the hypophysary-adrenocortical axis. In fact, GEORGES and HEROLD [467, 469] found that pre-treatment with 5-HT significantly inhibits ascorbic acid depletion produced in the rat adrenal glands by histamine, Pitressin®, adrenaline and formaldehyde.

Concerning the release of prolactin and gonadotropins see pages 252–253. On the whole, it would appear that 5-HT stimulates the liberation of prolactin, while inhibiting or leaving unaffected that of gonadotropins.

For an objective evaluation of the effects of 5-HT on the anterior pituitary one should not lose sight of the fact that the doses of the amine required to produce stimulation or inhibition are nearly always quite unphysiological [829].

According to LABORIT *et al.* [689] pituitary growth hormone increases the urinary excretion of 5-HIAA in the rabbit, thus demonstrating an interference with the metabolism of 5-HT.

This observation, together with the observation that 5-HT stimulates the growth of young plants and possesses anti-oxidant properties, is at the root of LABORIT's theory [688] that 5-HT is an essential growth factor favouring anaerobic processes. In the writer's opinion, speculations of this kind, lacking substantial experimental evidence, do not contribute to progress in 5-HT research.

Supraoptico-hypophyseal tract. Daily subcutaneous doses of 0.2 mg/kg 5-HT base administered for 7 days produce in rats:

- a) a decrease of the neurosecretory material in the posterior lobe of the hypophysis as well as in the cells of the supraoptic and paraventricular nuclei, with no change or even increase of the quantity of neurosecretory material going into the portal vessels in the region of the median eminence [660, 661];
- b) a moderate increase of the acetylcholinesterase and phosphatase activity of the hypothalamic magnocellular nuclei [659];
- c) an increase in the nucleus and nucleolus volume in the cells of the nucleus supraopticus [661].

KIVALO *et al.* conclude from the above findings that 5-HT stimulates the activity of the supraoptico-hypophyseal tract, and that 5-HT anti-diuresis seen in rats after 5-HT administration is due, at least in part, to the increased release of antidiuretic hormone.

Adrenals. ROSENKRANZ and LAFERTE [999, 1000] have reported that the *in vitro* addition of 5-HT (40–400 $\mu\text{g}/\text{kg}$ base per pair of adrenals) produces a release of a blue tetrazolium-reactive reducing material, possibly steroid in nature, from the adrenals of rabbits, guinea-pigs, rats and cows, as well as from a human adrenal tumour which appeared cortical in nature. The releasing-activity of 5-HT, which is protected by iproniazid but completely inhibited by amphenone, appears to be highly specific, as demonstrated by the failure of several other indolealkylamines, among which are 4-HT and 6-HT, to imitate the effect of 5-HT. The significance of this direct action of 5-HT on the adrenals is completely obscure.

It has been claimed that adrenalectomy produces in rats an increase of the 5-HT levels in skin, ears, liver, lung [576] and brain [319], a progressive increase (up to 70%) of the urinary excretion of 5-HIAA [358b] and a decrease of the concentration of 5-HT in serum. However, the influence of acute bilateral adrenalectomy on brain 5-HT could not be confirmed [1205].

Glucocorticoids have been found to lower the 5-HT level in the skin and small intestine of normal rats (in spleen no 5-HT depletion was noted and the stomach presented, instead of a decrease, an increase in the amine content), to retard the recovery of 5-HT in the skin depleted of the amine by prolonged treatment with polymyxin, and to restore to normal the increased 5-HT concentration seen in the skin of adrenalectomized rats. Mineralocorticoids are inactive [576, 1304, 1196b].

TELFORD and WEST [1196b] suggest that the tissue reserves of 5-HT and histamine may be in part regulated in rats by the secretion of the adrenal cortex.

Thyroid. 2–3 mg/kg 5-HT produces in rats a decrease in thyroid activity, as studied by means of radio-active iodine. This effect is enhanced by iproniazid [1354].

6.15 Miscellaneous effects

6.151 Protective effect against X-radiation

Premedication with 5-HT reduces acute lethality in rats and mice following irradiation, as well as incidence of diarrhoea and severity of depilation in survivors [44, 1245, 1246, 823, 680, 692, 976, 770, 968b].

The prophylactic threshold dose in mice is 20 mg/kg, i.p. [823], in rats less than 15 mg/kg [1245, 680]. Protection afforded by 5-HT given after exposure to X-rays is very low [823]. Similarly, an increase in respiratory oxygen pressure to 4–5 atmospheres almost completely reverses the protective action of 5-HT in rats [1245].

5-HTP is more than 10 times less effective than 5-HT, 5-methoxytryptamine shows a very limited protective action, and bufotenine and bufotenidine are inactive [976].

BOL and BAS-phenol antagonize the protective effect of 5-HT, whereas antihistamines have no effect. Tranquillization with reserpine does not significantly influence lethality [1246].

6.152 Action on cellular permeability

This has been investigated in three representative cell models.

Slices of red beetroot. 5-HT, indole-acetic acid and, to a lesser degree, tryptamine (1–100 $\mu\text{g/ml}$) increase the effusion of pigment from beetroot slices, and strongly inhibit sodium uptake by this material, although they display little effect on the potassium efflux. There is no appreciable change in the rate of oxygen consumption of this tissue [923, 922].

Erythrocytes. Pre-treating human erythrocytes with 5-HT (0.1–1 mM) accelerates their subsequent hypotonic haemolysis as shown by the haemoglobin release, but diminishes the amount of potassium lost in proportion to haemoglobin. Similarly, 5-HT pre-treated erythrocytes lose potassium more slowly in cold-storage than do controls. Tryptamine in similar concentrations does not have either of these effects. LSD (0.1–1 mM) inhibits the effects of 5-HT [924, 925, 926].

Isolated amphibian skin. Addition of 5-HT (15–400 $\mu\text{g/ml}$ of the base) to water used to bathe the outer surface of isolated abdominal frog or toad skin produces no significant effect on the net water flux, while increasing the net fluxes of sodium and potassium. Tryptamine has effects similar to those of 5-HT, whereas bufotenine is practically inactive.

It has been suggested by PICKLES [927, 928] that the above effects may be explained by a selective alteration in cell-membrane permeability and that this may be relevant to the problem of the physiological function of 5-HT.

6.153 Action on the gill cilia of lamellibranchiates

Both 5-HT (threshold concentration 0.1 $\mu\text{g/ml}$, optimum concentration 100 $\mu\text{g/ml}$) [510] and 5-HTP (5–100 $\mu\text{g/ml}$) [833] stimulate the activity of cilia on excised gills of lamellibranchiate molluscs.

Beat frequency of the cilia and velocity of the metachronal wave increases while the average wave length remains unchanged [7]. The action of 5-HT is more prompt but that of 5-HTP lasts longer, up to several hours [833].

LSD and BOL block the action of 5-HT, but at the same time are effective accelerators of gill cilia [510].

Since gills of lamellibranchiates contain small amounts of 5-HT or a 5-HT-like substance, it has been suggested that endogenous 5-HT is concerned in the physiological regulation of ciliary activity [833, 7].

6.154 Acute toxicity of 5-HT

The following agents have been found to increase the acute toxicity of 5-HT:

a) *pertussis vaccine.* Injections of pertussis vaccine produce in mice, independently of their strain, an increased sensitivity to 5-HT. This appears 24

hours after the injection of the vaccine, reaches its peak after 3–4 days, and disappears within 25 days. Ability to induce 5-HT sensitivity seems to be characteristic of *H. pertussis* [853, 854, 629].

b) *iproniazid*. Pre-treatment with iproniazid increases the toxic effects of 5-HT in rats and mice, as well as the ability of the amine to produce lenticular cataracts [149].

c) *48/80*. LD 50 of 5-HT base, given by the intravenous route, decreases in mice from the normal value of 137 mg/kg to 26.5 and 4.2 mg/kg, following pre-treatment with 1 and 1.5 mg/kg 48/80, respectively [577].

d) *Enosine*. This drug in mice deepens 5-HT sedation and increases 5-HT toxicity [509].

7. 5-HT Like Substances

As a direct consequence of the enormous interest raised by 5-HT and the other naturally occurring indolealkylamines a number of related indole derivatives has been synthesized and studied.

Detailed results of these studies have been reported repeatedly in this review article. Here we wish only to summarize in Tables 12 and 13 the relative activities of the most important indolealkylamines and indazolealkylamines, as assessed on some of the smooth muscle preparations most commonly used in the bioassay of 5-HT.

The following indole aminoacids and acids have proved to be inactive or at least 10,000 times less active than 5-HT on the smooth muscle preparations employed: L-tryptophan, 5-HTP, 4-HTP, 6-HTP, 7-HTP, 2-HTP, 5-hydroxy-N-acetyl-TP, 5-acetoxy-N-acetyl-TP, 5-hydroxy-N,N-dimethyl-TP, 5-hydroxy-2-methyl-TP; IAA, 5-HIAA, 4-HIAA, 7-HIAA, 6-HIAA and 5-methoxy-IAA.

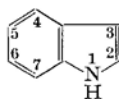
It may easily be seen from the tabulated data that no general conclusion is possible in regard to the relationship between chemical constitution and pharmacological activity. Therefore discussion will be limited to some of the data collected on the rat uterus and rat stomach preparations, which behave in a fairly similar manner:

a) Shifting of the phenolic -OH group from position 5 to other positions regularly produces a decay of biological activity, which is moderate when the transposition is to position 4, but tremendous when the transposition is to positions 6 or 7.

b) Substitution on the indole nucleus of groups other than -OH groups regularly reduces biological activity. The reduction is moderate in the case of amino- and methoxy-groups, but considerable in the case of halo-groups and benzyloxy-groups.

c) Etherification of the phenolic -OH with glucuronic or sulphuric acid practically abolishes biological activity; etherification of the phenolic -OH with phosphoric acid does not provoke any change in activity in the case of psilocin and bufotenine (see als WEIDMANN and CERLETTI [1280]).

Table 12



Equipotent molar ratios of indolealkylamines estimated on five isolated smooth muscle preparations. 5-HT taken as unity

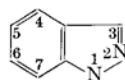
No.	Ring substituent	Side-chain	Stimulant activity				
			Rat uterus [100, 68, 69]	Rat stomach [68, 69, 1247]	Molluscan * heart [100, 522]	Rabbit ear [100]	Guinea-pig ileum [100]
I	5-OH	3-CH ₂ -CH ₂ -NH ₂ (5-HT)	1	1	1	1	1
II	5-OH	3-CH ₂ -CH ₂ -NHCH ₃	1	6.3	=	1.4	7
III	5-OH	3-CH ₂ -CH ₂ -N(CH ₃) ₂	4	10	0.3 (0.028)	11	4
IV	5-OH	3-CH ₂ -CH ₂ -N(C ₂ H ₅) ₂	9	18; 20	=	=	=
V	5-OH	3-CH ₂ -CH ₂ -N(C ₃ H ₇) ₂	39	16; 4	=	=	=
VI	5-OH	3-CH ₂ -CH ₂ -N(C ₃ H ₇ iso) ₂	16	4.5	=	=	=
VII	5-OH	3-CH ₂ -CH ₂ -N(C ₄ H ₉) ₂	540; 1100	151	0	0	450
VIII	5-OH	3-CH ₂ -CH ₂ -N(CH ₃) ₃	>1000[364]	=	=	=	=
IX	5-OH	3-CH ₂ -CH(CH ₃)-NH ₂	1.3; 2	1.4; 1.7	8.2 (6)	3.3	8
X	5-OH	3-CH ₂ -C(CH ₃) ₂ -NH ₂	310	=	172	0	24
XI	5-OH	3-CH ₂ -CH(C ₂ H ₅)-NH ₂	50	7	50	40	44
XII	5-OH	3-CH ₂ -CH ₂ -NHCOCH ₃	+ - [809]	=	=	=	=
XIII	5-OH	3-CHOH-CH ₂ -N(CH ₃) ₂	0	=	0	0	0
XIV	5-OH	3-CH=CH-NH ₂	>1000[364]	=	=	=	=
XV	5-OH	3-CH ₂ -C(N)-NH ₂	0	=	=	108	=
XVI	5-OH	2-CH ₂ -CH ₂ -NH ₂	0	=	0	0	190
XVII	5-OCH ₃	3-CH ₂ -CH ₂ -NH ₂	9.6; 8	20; 39	0.5	5.4	9
XVIII	5-OCH ₃	3-CH ₂ -CH ₂ -N(C ₄ H ₉) ₂	0	=	540	0	0
XIX	5-OCH ₃	3-CH ₂ -CH(CH ₃)-NH ₂	1.5; 4.3	2.9; 1.4	4.6	1.5	43
XX	5-OCH ₃	3-CH ₂ -CH ₂ -NHCOCH ₃	0	=	=	345	0
XXI	5-CH ₃	3-CH ₂ -CH ₂ -NH ₂	173	184; 620	=	=	=
XXII	5-CH ₃	3-CH ₂ -CH(CH ₃)-NH ₂	1300; 240	14; 22	190	340	0
XXIII	5-Cl	3-CH ₂ -CH ₂ -NH ₂	=	100	=	=	=
XXIV	5-NH ₂	3-CH ₂ -CH ₂ -NH ₂	11	=	1.3	23	22
XXV	5-OC ₇ H ₇	3-CH ₂ -CH ₂ -N(CH ₃) ₂	20000	625	=	=	=
XXVI	5-OC ₇ H ₇	3-CH ₂ -CH ₂ -N(C ₂ H ₅) ₂	10000	470	=	=	=
XXVII	5-OC ₇ H ₇	3-CH ₂ -CH ₂ -N(C ₃ H ₇) ₂	20000	614	=	=	=
XXVIII	5-OC ₇ H ₇	3-CH ₂ -CH ₂ -N(C ₄ H ₉) ₂	20000	20000	=	=	=
XXIX	5-OC ₇ H ₇	3-CH ₂ -CH(CH ₃)-NH ₂	20000	63	=	=	=
XXX	5-O-sulphate	3-CH ₂ -CH ₂ -NH(CH ₃) ₂	500	=	=	0	1000
XXXI	5-O-sulphate	3-CH=CH-N(CH ₃) ₂	>1000[364]	=	=	=	=
XXXII	5-O-glucuronide	3-CH ₂ -CH ₂ -NH ₂	0	=	0	=	0
XXXIII	5-O-glucuronide	3-CH ₂ -CH ₂ -N(CH ₃) ₂	0	=	0	=	0

Table 12 (continued)

No.	Ring substituent	Side-chain	Stimulant activity				
			Rat uterus [100, 68, 69]	Rat stomach [68, 69, 1247]	Molluscan * heart [100, 522]	Rabbit ear [100]	Guinea-pig ileum [100]
XXXIV	5-OH, 2-CH ₃	3-CH ₂ -CH ₂ -NH ₂	=	=	(31.4)	=	=
XXXV	5-OCH ₃ , 2-CH ₃	3-CH ₂ -CH ₂ -NH ₂	=	=	(43.8)	=	=
XXXVI	5, 6-(OCH ₃) ₂	3-CH ₂ -CH ₂ -NH ₂	58	300	4	144	300
XXXVII	5, 6, 7-(OCH ₃) ₃	3-CH ₂ -CH ₂ -NH ₂	110	=	130	560	145
XXXVIII	6-OH	3-CH ₂ -CH ₂ -NH ₂	0	460	47	155	0
XXXIX	6-OCH ₃	3-CH ₂ -CH ₂ -NH ₂	0	1520	174	480	400
XL	4-OH	3-CH ₂ -CH ₂ -NH ₂	11	1.8	15	22	26
XLI	4-OH	3-CH ₂ -CH ₂ -N(CH ₃) ₂	1000 [101]	=	=	=	=
XLII	4-O-phosphate	3-CH ₂ -CH ₂ -N(CH ₃) ₂	330	=	18	0	760
XLIII	7-OH	3-CH ₂ -CH ₂ -NH ₂	0	=	0	1300	0
XLIV	2-CH ₃	3-CH ₂ -CH ₂ -N(CH ₃) ₂	20000	1200; 6600	=	=	=
XLV	2-CH ₃	3-CH ₂ -CH ₂ -N(C ₂ H ₅) ₂	1000	280	=	=	=
XLVI	2-CH ₃	3-CH ₂ -CH ₂ -N(C ₃ H ₇) ₂	630	70	=	=	=
XLVII	1-CH ₃	3-CH ₂ -CH ₂ -NH ₂	142	=	0	330	520
XLVIII	1-CH ₃	3-CH ₂ -CH(CH ₃)-NH ₂	1356	69	=	=	=
XLIX		3-CH ₂ -CH ₂ -NH ₂	176; 210	408; 933	22 (9.9)	20	280
L		3-CH ₂ -CH ₂ -NHCH ₃	128; 2000	1120; 2400	(3.7)	17	48
LI		3-CH ₂ -CH ₂ -NHC ₂ H ₅	2000	250; 350	(9.1)	=	=
LII		3-CH ₂ -CH ₂ -NHC ₃ H ₇	0; 2000	330; 490	0	0	0
LIII		3-CH ₂ -CH ₂ -N(CH ₃) ₂	200; 1000	196; 350	107 (10.7)	35	58
LIV		3-CH ₂ -CH ₂ -N(C ₂ H ₅) ₂	160; 500	83; 112	650 (7.9)	0	0
LV		3-CH ₂ -CH ₂ -N(C ₃ H ₇) ₂	95; 200	34; 40	59	0	0
LVI		3-CH ₂ -CH ₂ -N(C ₃ H ₇ iso) ₂	520	53	=	=	=
LVII		3-CH ₂ -CH ₂ -N(C ₄ H ₉) ₂	720; 500	244	810	0	870
LVIII		3-CH ₂ -CH(CH ₃)-NH ₂	0; 800	31; 50	0 (8.6)	0	0
LIX		3-CH ₂ -CH(C ₂ H ₅)-NH ₂	20000	4600	=	=	=
LX		3-CH ₂ -C(CH ₃) ₂ -NH ₂	=	1400	=	=	=
LXI		3-CHOH-CH ₂ -NHCH ₃	0	=	23	370	0
LXII		3-CH ₂ -C(N)-NH ₂	0	=	=	120	=
LXIII		3-CH ₂ -CH ₂ -CH ₂ -NH ₂	=	1920	=	=	=
LXIV		3-CH ₂ -CH ₂ -CH ₂ -N(CH ₃) ₂	=	=	(2000)	=	=
LXV		3-CH ₂ -NH ₂	=	29000	=	=	=
LXVI		3-CH ₂ -N(CH ₃) ₂	0	=	0 (0)	0	400

* Values outside of parentheses have been obtained on the heart of *Helix lucorum* [100], values in parentheses on the heart of *Venus mercenaria* [522].

Table 13



Equipotent molar ratios of indazolealkylamines estimated on four isolated smooth muscle preparations [100]

5-HT taken as unity

No.	Ring substituent	Side-chain	Stimulant activity			
			Rat uterus	Helix heart	Rabbit ear	Guinea-pig ileum
1	5-OH	5-hydroxytryptamine	1	1	1	1
2	5-OH	3-CH ₂ -CH ₂ -NH ₂	4, 3-5 [9]	3.9	27	5.3
3	5-OC ₇ H ₇	3-CH ₂ -CH ₂ -NHCH(CH ₃) ₂	227	172	0	0
4	5-OC ₇ H ₇	3-CH ₂ -CH ₂ -NH ₂	0	0	0	0
5	5-OC ₇ H ₇	3-CH ₂ -CH ₂ -N(CH ₃) ₂	0	0	0	0
6	5-OC ₇ H ₇	3-CH ₂ -CH ₂ -NHCH(CH ₃) ₂	0	0	0	0
7		3-CH ₂ -CH ₂ -NH ₂	360	335	134	0
8		3-CH ₂ -CH ₂ -N(CH ₃) ₂	39	7.2	85	20
9		3-CH ₂ -CH ₂ -NHCH(CH ₃) ₂	0	0	0	0
10		2-CH ₂ -CH ₂ -NH ₂	0	0	700	570
		1-CH ₂ -CH ₂ -NH ₂	870	0	66	185

d) Lack of any phenolic -OH group, as in tryptamine, is accompanied by a strong reduction in biological activity which, however, is much less than that observed following substitution of an -OH group at position 6 or 7 of the indole nucleus.

e) Shifting of the side chain from position 3 to position 2 produces a complete loss of biological activity.

f) The effect of alkyl substitution on the amino nitrogen is variable; in general both an increase in number and size of the alkyl groups produces a reduction of biological activity.

g) Substitution of a methyl group on the α -carbon of the side chain, i.e. introduction of a third carbon atom into the side chain, produces either a moderate decay of activity or an increase in activity, depending upon other substituents in the molecule. Substitution of two methyl groups or of one ethyl group is considerably more deleterious.

h) In sharp contrast to what usually happens with phenylalkylamines, substitution of an -OH group on the β -carbon of the side chain produces the practical disappearance of any activity on the rat uterus preparation.

i) Indolealkylamines with a single carbon atom side chain (gramine derivatives) show a negligible activity on all preparations tested.

k) Indazolealkylamines show a remarkable 5-HT-like activity, which is particularly evident for the first member of the series, more strictly related to 5-HT from a chemical point of view.

VANE [1247] has observed that the relative potency of many of the examined indolealkylamines changes considerably following introduction of a MAO inhibitor into the nutrient bath of the isolated rat stomach. He suggests that MAO block increases the potency of those compounds which more easily enter the cells where MAO is active.

The tabulated data show that the molluscan heart not rarely gives results which differ strikingly from those obtained with the rat uterus or stomach preparations. This fact should not be overlooked when submitting to bioassay crude extracts from vegetable or animal tissues. The presence, for example, of N-alkylated indolealkylamines would greatly impair the accuracy of estimation of 5-HT.

8. Morphological Changes Produced by 5-HT and 5-HTP

8.1 Blood

Repeated intraperitoneal or subcutaneous administration of large doses of 5-HT (20–120 mg/kg 5-HT base, daily for 20–60 days), produces in rats a 3- to 4-fold increase in platelet count without any other significant change in the blood picture [183, 563]. Under the same conditions, the increase in the number of platelets is considerably less in the guinea-pig [604].

Daily administration, for periods up to 12 months, of 200 mg/kg 5-HTP does not cause in rats any change in platelet count [304].

Single large doses of 5-HT (20–50 mg/kg) produce in rats a 40–60% increase in the blood platelets, lasting 24 hours, and a definite decrease in the eosinophile count. Doses of 2 mg/kg are ineffective [1173, 1089]. Following adrenalectomy the platelet increase persists but, instead of decreasing, the number of eosinophiles also rises [1174]. In iproniazid-pretreated rats the duration of the eosinopenia due to 5-HT is slightly prolonged and the typical increase in thrombocytes fails to appear. 5-HTP (27 mg/kg, i.v.) causes eosinopenia but not thrombocytosis; 5-HIAA (50 mg/kg, i.v.), on the contrary, causes eosinopenia as well as thrombocytosis [1348].

In the rabbit a single parenteral dose of 5-HT (6–18 mg/kg) produces a transient, definite increase in the thrombocytes and leucocytes [407]; after 24 hours the increase specifically regards the basophil leucocytes [151].

In man the most striking acute effect of 5-HT (4–12 mg) is to reduce transiently circulating eosinophil leucocytes. This effect is inhibited by antihistamines [240].

Results obtained in rats and guinea-pigs are, on the whole, rather confused and, owing to the absolutely unphysiological, toxic dosage of 5-HT used, it is difficult to ascribe any particular significance to them.

8.2 Kidney

Nephrotoxicity of 5-HT in rats was first observed by ERSPAMER [364], and then confirmed by numerous other investigators [888, 1031, 56, 418, 757, 565].

Renal lesions produced by single or multiple injections of 5-HT, in doses of 1–20 mg/kg or more, consist essentially of coagulative necrosis of the proximal convoluted tubules and/or conspicuous tubular dilation. The lesions, which are mainly focal in distribution, result either in complete repair or in fibrotic scarring, according to their severity. A nephrotoxic action identical with that possessed by 5-HT has also been shown by 5-HTP and 4-HTP. Combination of noradrenaline and 5-HT produces more severe and extensive lesions than 5-HT alone.

Concomitant administration of hydralazine, chlorpromazine, levopromazine, Dibenamine®, dicyclomine, LSD, BOL or sodium glutamate with 5-HT effectively prevents lesions; treatment with testosterone provides some protection, whereas estradiol exerts an aggravating action [178, 618].

It has been suggested that cortical necrosis from 5-HT is due to ischemic periods consequent to arterial spasm probably affecting interlobular and afferent glomerular arteries.

The powerful antidiuretic effect of 5-HT in the rat has been described in detail in the preceding chapter. Here we wish to lay emphasis on the fact that the above gross renal lesions, seen after mg doses of 5-HT, are strong evidence in favour of the view that 5-HT antidiuresis is brought about through a direct renal vascular mechanism.

Although rat kidney is particularly sensitive to 5-HT, the amine may produce renal lesions in other species, too. Thus rabbits given daily subcutaneous doses of 20 mg/kg 5-HT base for 8 months, exhibit sclerolalinos of the glomeruli and adenomatous hyperplasia of the tubules [1257].

However, in spite of the potential nephrotoxicity of 5-HT, it does not appear that the amine plays any role in the oliguria subsequent to thermal burns [975]. Similarly it is highly questionable whether renal lesions or even changes in renal haemodynamics resulting from blood trauma in extracorporeal circuits may be attributed to 5-HT, as suggested by some investigators [1053, 1054, 443].

8.3 Gastro-intestinal tract

5-HT and 5-HTP, when injected in sufficiently large doses, cause in rats and mice the appearance of haemorrhagic mucosal erosions in the stomach and the colon evolving, after prolonged treatment, into chronic ulcers and scars [565, 227, 559, 1067, 124, 1315, 1314].

Vascular spasms seem to play a major pathogenetic role in the production of these lesions [124].

Following two subcutaneous doses of 15 mg/kg 5-HT, 95% of the rats exhibit ulcers in the stomach and 50% in the colon; following two doses of 300 mg/kg 5-HTP the percentages are 40 and 0, respectively [1315, 1314].

Tryptamine (500 mg/kg $\times$ 2), tryptophan, iso-5-HT (100 mg/kg $\times$ 2) and iso-5-HTP (300 mg/kg $\times$ 2) do not display any ulcerogenic effect [1314].

LSD, UML, chlorpromazine and atropine effectively prevent gastric lesions produced by 5-HT and 5-HTP, whereas iso-5-HT, promethazine, thenalidine

and papaverine are considerably less active [1315, 1314, 555, 863]. Histamine enhances the pathological effects of 5-HT [618].

In mice 5-HT fails to produce gastric haemorrhage when administered to vagotomized animals or to animals pre-treated with iproniazid [124].

It is possible that, as with renal lesions, so may gastric lesions produced by 5-HT heal with *restitutio ad integrum* in spite of continuous administration of the drug. In fact, MACDONALD *et al.* [757] and DAVIDSON *et al.* [863] have failed to detect ulcers of the gastro-intestinal tract in rats treated up to 353 days with daily subcutaneous doses of 7 mg 5-HT base/rat, or daily intraperitoneal doses of 200 mg/kg 5-HTP.

Owing to the 5-HT-releasing activity of reserpine, it has been repeatedly suggested [124, 863] that gastric ulcers provoked by this drug might be due to 5-HT. Experimental evidence is decidedly against this view [559, 686], and it should be noted that 5-HT seems to enhance rather than depress resistance of the gastric mucosa to ulcer promoting influences [1349, 732, 701].

8.4 Submaxillary glands

Necrosis of the submaxillary glands has been found in rats to be almost as constant as kidney injury, following the intraperitoneal injection of 40 mg/kg 5-HT base. Necrosis begins at the inferior pole and usually extends over the lower half of the gland. It is characterized by a total destruction of the acini. No lesions have been found in the other salivary glands [618].

8.5 Liver

In young rats a single subcutaneous injection of 16 mg/kg or more 5-HT base results in increased formation of hepatic parenchymal cells and an increased number of mitotic figures. In no instance, even with extremely large doses of 5-HT, has frank cellular necrosis been found [759].

5-HT does not influence fatty liver, leading to cirrhosis, when produced with a choline-deficient, high-fat, high-caloric diet [757]; however, high doses of the drug (4–12 mg/kg of the base) reduce the liver damage provoked by allyl alcohol [351] and protects rats against both initial liver damage [420] and cirrhosis caused by carbon tetrachloride [422, 423b].

FIGORE DONATI *et al.* [422] suggest that the hepatoprotective effect of 5-HT may be due to liberation of ACTH and adrenocortical steroids.

8.6 Cardio-vascular system

The action of chronic administration of 5-HT on the right heart and on the pulmonary artery has been studied with particular care in order to throw light on the pathogenesis of the cardio-vascular lesions seen in carcinoidosis.

Results obtained in experimental animals have not provided decisive evidence in favour of the otherwise well founded hypothesis that excess circulating

5-HT is directly responsible for the valvular and vascular changes observed in carcinoid patients.

Rat. Chronic administration of large doses of 5-HT (4–16 mg/kg/day) for periods of 12–16 months at first produces intense perivascular oedema, affecting a number of arteries, and then severe perivascular fibrosis [1064, 349].

A reduction of the metachromatic substance has been noted in the large arteries [233].

Cardiac valvular changes do not occur, as a rule [757, 756, 760, 419]; however, in some instances, after 12 months' treatment, an increase of the connective tissue in the right-heart valves has been described [349].

Daily intraperitoneal injections for periods of up to 12 months of 5-HTP in doses as large as 200 mg/kg have failed to produce any significant alteration of the heart [304], thus further demonstrating the relative harmlessness of 5-HT to the rat heart.

Rabbit. Chronic administration of 5-HT (1 mg/kg/day) for 50 days produces in the medium and small arteries various degrees of fibrous-elastic intimal hyperplasia as well as mild hypertrophy of the muscular wall. Deposits of mucopolysaccharides occur often in arteries with intimal lesions [1002].

Whereas with the above dosage of 5-HT no lesions in the pulmonary vessels have been noted, at higher dose levels (8 mg/kg/day up to 8 months) the pulmonary artery has shown a fibrous-elastic intimal hyperplasia with interstitial oedema and lesions of the muscular layer [1257].

Dog. Multiple subcutaneous injections of 5-HT (2 mg/kg/day), for periods of 4 to 5 months cause in the dog marked alterations of the pulmonary vessels, such as hyperplasia of the muscular and elastic layers with adventitial and peri-adventitial sclerosis, especially affecting the medium-sized and small arteries [225].

8.7 Skin

It has been regularly noted in all species examined, that repeated subcutaneous administration of 5-HT in sufficiently high concentrations produces dermal fibrosis at the site of injections. At the same time, necrosis of the tips of the tail and of the digits and opacities of the lens of the eyes often occur in rats [757].

It is considered that the above lesions are the result of the vasoconstrictor and ischemic effects of 5-HT.

Single or repeated intradermal injections of 2 mg 5-HT regularly produce a small degree of oedema in the rabbit skin, but no eosinophil infiltration has been seen [29].

8.8 Sex organs

Mammary gland. Daily injections of 2–4 mg/kg 5-HT for 5–20 days induce in rats—like adrenaline, reserpine and acetylcholine—mammary growth and lactation. Lobulo-alveolar development is particularly evident if 5-HT is given

after estradiol priming. In *post partum* rats the amine injected after litter removal retards mammary involution and maintains secretory function.

No effect has been observed in animals hypophysectomized after estrogen-priming. It has been suggested that 5-HT elicits its mammary effect through a discharge of prolactin from the pituitary [820, 822, 821, 952].

Other sex organs. In immature female mice, 1–25 mg/kg 5-HT daily for 2–5 weeks, produces little or no effect on body weight. However marked depression of the growth of the ovaries, uterus and vagina, as well as a delay in the opening of the vagina and in the onset of vaginal oestrus has been observed.

In mature mice daily doses of 20–25 mg/kg 5-HT markedly depress the weight of sex organs [154, 987b]. However, the action of the amine on the oestrus cycle and ovulation is ambiguous [320, 327].

The weight of the preputial glands has been found to increase following a single subcutaneous dose of 4 mg/kg 5-HT. The increase is less evident in ovariectomized and ovariectomized-adrenalectomized rats [421].

9. Participation of 5-HT in Anaphylaxis, Inflammation and Response to Stress

9.1 Anaphylaxis

9.1.1 Mouse

FINK [416] first suggested that the contraction of the egg-white sensitized mouse uterus produced by addition of antigen might be dependent on a release of 5-HT from the uterine tissue. In fact, the reaction is always completely prevented by LSD or reserpine.

The results and conclusions of FINK are generally accepted in spite of the fact that the above LSD inhibition is not reproducible in the rat, that direct evidence of a 5-HT release from mouse uterus is completely lacking and that there is a wide divergence of opinion in regard to the role of 5-HT in the general anaphylactic reaction of the mouse.

The findings of TOKUDA and WEISER [1201] that pretreatment with 5-HT antagonists such as BOL, LSD and chlorpromazine, or 5-HT depletors such as reserpine, protects mice against anaphylactic shock, that the refractoriness of reserpinized animals is reversed by treatment with 5-HTP but not with DOPA and, finally, that prechallenge treatment with 5-HT or 5-HTP increases the susceptibility of the animals to anaphylaxis are positive pieces of evidence in favour of the 5-HT theory of anaphylaxis. However other observations do not support this theory.

MUNOZ [854], for example, has been unable to confirm the protective action of either LSD or BAS (the latter product seems to encourage anaphylactic death). HIGGINBOTHAM [578] reports that adrenalectomy decreases resistance of mice to anaphylaxis more markedly than to 5-HT (100 times versus 6 times) and that anaphylactic phenomena are more resistant to cortisone therapy than

5-HT intoxication. WAALKES and COBURN [1271] have failed to detect any significant change in the blood or tissue levels of 5-HT following anaphylactic shock, whereas levels of histamine rise in the lung. Finally, DONALDSON *et al.* [336] have shown that in mice bearing mastocytomas neither the tumour nor the conspicuous quantity of 5-HT released from kidney, lung, small intestine and liver during the shock has any effect on the course of anaphylaxis.

9.12 Rat

BROCKLEHURST [165] could not confirm that 5-HT is responsible in rats for the anaphylactic contraction of the uterus isolated from animals sensitized passively to ovalbumin.

In fact, the contraction persists in the presence of large amounts of LSD, and in the absence of the antagonist the sensitivity of the tissue to 5-HT is greater shortly after anaphylactic shock than previously, which is in contrast to the known tachyphylaxis produced by large doses of 5-HT.

Moreover, BROCKLEHURST *et al.* [166] could not demonstrate—following pre-treatment with 5-HT and histamine antagonists or releasers—any significant change either in the reversed passive cutaneous anaphylactic reactions or in the direct cutaneous anaphylactic reactions produced in passively and actively sensitized rats. They conclude that histamine and 5-HT are not primary mediators of anaphylactic reactions in the rat skin but play only a secondary part.

Exactly the same results have been obtained by SANYAL and WEST [1049] who have reported that severity of the symptoms seen in anaphylactic shock remains unchanged in the presence of 5-HT and histamine antagonists, or following amine depletion.

Finally, MOTA [848] is against the participation of 5-HT in rat anaphylaxis because of the absence in the plasma of shocked rats of any activity that could not be inhibited by antihistamines and of the protection afforded by these drugs against the shock.

However, GARCIA-ARROCHA [462] is of a partly divergent opinion.

Having observed that incubation, with the antigen, of minced uterine tissue taken from rats sensitized to horse serum results in a release of substantial quantities of 5-HT but no detectable histamine, and that both histamine and 5-HT are liberated by the antigen from perfused small intestine and isolated peritoneal fluid cells, the above investigator suggests that the anaphylactic response of the rat uterus depends on the release of 5-HT, whereas both histamine and 5-HT may participate in the anaphylactic response of other rat tissues.

Haemophilus pertussis vaccine aggravates anaphylactic shock in the rat, the target organ being the small intestine. Histamine and 5-HT do not seem to play major roles in the reaction [1048].

Similarly, neither 5-HT nor histamine seem to mediate the severe cutaneous inflammation which develops on challenging, with *H. pertussis* vaccine, the skin of rats pretreated 6 to 28 days previously with the same vaccine [1011].

9.13 Guinea-pig

The great majority of investigators share the opinion that 5-HT plays only an unimportant role, if any, in the systemic or local anaphylactic reaction of the guinea-pig [343, 397, 417, 569, 570, 611, 1047, 1050, 737, 1284].

This opinion, however, is not entirely accepted by others, on the basis of the fact that LSD [893] or azulen [484], given prior to serum, afford significant protection against fatal anaphylaxis and that a combination of LSD with anti-histamines and atropine is required to suppress completely the Schultz-Dale reaction in the guinea-pig uterus [465] and, finally, that 5-HT is released from intestine and spleen of guinea-pigs during anaphylactic shock [359].

9.14 Rabbit

5-HT is released from the rabbit platelets during anaphylactic shock [606, 1264, 1265], as shown by the immediate rise in plasma 5-HT occurring after the injection of the specific antigen [1264], and by the increase in urinary excretion of 5-HIAA [426].

Furthermore, during anaphylaxis the 5-HT of whole blood shows a sharp drop (from 3.2–6 μg to 0.8 $\mu\text{g}/\text{ml}$) whereas the lungs show a formidable increase in 5-HT content (from 3 to 22 $\mu\text{g}/\text{g}$), as a consequence of the disappearance of the platelets which are held in the lung [1268].

Pretreatment of the animals with heparin (3000 I. U./kg, i.v.) prevents the above effects and also the release of 5-HT from platelets in sensitized blood after addition of the specific antigen. Similarly, heparin inhibits the ability of platelets to absorb 5-HT *in vitro* [620b].

In spite of the observed changes in 5-HT distribution, the amine is generally believed to be, in the rabbit too, of minor importance in the pathogenesis of the anaphylactic reaction and of allergic vascular lesions [711, 264].

In fact, depletion of tissue 5-HT produced by reserpine does not prevent the fatal shock provoked by egg-white, nor is the shock evidently antagonized by LSD, Hydergine® or chlorpromazine [710, 709].

This does not, however, rule out the possibility that 5-HT may participate in some of the toxic phenomena seen in rabbit anaphylaxis.

MARCELLE and LECOMTE [788], for example, suggest—but the low sensitivity of the rabbit intestine towards 5-HT is against this suggestion—that the intestinal hyperperistalsis seen in rabbits during anaphylactic shock may be due to liberation of 5-HT; WEISSBACH *et al.* [1284] believe that both 5-HT and histamine should be considered in explaining the pulmonary effects seen in anaphylaxis.

Changes in the 5-HT content of blood and organs similar to those provoked by intravenous injection of the antigen into sensitized rabbits are also caused by intravenous injection of the antigen-antibody precipitate into normal rabbits, thus demonstrating that results seen in sensitized rabbits are mediated by this complex. It is of interest that even intravenous injections of glycogen (100 mg/kg) cause in rabbits a marked fall in whole blood 5-HT concentration and

a rise in lung content of the amine, secondary to trapping of platelets in the lung [1270].

Because the *Arthus phenomenon* develops readily in rabbits depleted of 5-HT by reserpine, it appears doubtful that this amine is of importance in the production of the lesions [1272].

9.15 Dog

5-HT does not seem to play any important role in the anaphylactic reaction of the dog [1050]. However, participation of 5-HT in the bronchospasm produced in sensitized animals by antigen administration has been suggested [865].

9.16 Man

HERXHEIMER and SCHACHTER [571] believe that it is unlikely that the release of 5-HT is of significance in reactions involving whealing or in angio-oedema reactions in man. In fact, 5-HT, which is an effective flaring agent when given intradermally in man, fails to cause whealing or other evidence of increased capillary permeability.

9.2 Anaphylactoid reaction

Systemic and/or local administration of egg-white, ovomucoid, dextran and 48/80 produce hyperemia and oedema of paws and snout in rats. This is the so-called anaphylactoid reaction.

It has been suggested that this reaction is mediated mainly by 5-HT, or by both 5-HT and histamine or, finally, by a more complex mechanism involving amines and other ill-defined substances.

Sustainers of the 5-HT theory lay stress on the fact that

a) subcutaneous 5-HT is about 200 times more potent than histamine in producing oedema, doses as low as 0.2–0.3 μg being effective;

b) 5-HT produces oedema both in the normal animal and in the animal whose skin histamine has been previously depleted by treatment with compound 48/80 or with polymyxin B;

c) the oedema fluid contains detectable amounts of 5-HT, but no histamine [904, 905, 908, 910, 909];

d) no oedema is produced if the skin is first depleted of its 5-HT content by reserpine treatment or prolonged treatment with compound 48/80 [908, 909, 910, 815, 36, 846];

e) the anaphylactoid reaction is prevented by previous administration of BOL or LSD [815, 36, 846], but is only slightly modified by anti-histaminic substances [908, 909, 910];

f) the areas more prone to the production of increased capillary permeability by 48/80 are rich in both histamine and 5-HT. Other areas containing histamine but less 5-HT exhibit this response weakly [910];

g) subcutaneous injection into the rat paw of dextran or turpentine oil produces a significant reduction (from 0.75 to 0.22–0.28 $\mu\text{g/g}$) of the 5-HT content not only in the injected paw but also in the opposite untreated paw [1183b].

Sustainers of the theory that multiple agents are involved in the pathogenesis of the anaphylactoid reaction object that substances which produce this reaction release 5-HT as well as histamine from skin tissues [908, 847, 539];

that higher doses of BOL or LSD are required to block anaphylactoid reaction than to block oedema produced by 5-HT [847b];

that while Dibenamine® regularly inhibits the oedema produced by 5-HT, and pyrilamine that produced by histamine, both drugs are necessary to block the oedema-producing action of ovomucoid [1010];

that pretreatment with the MAO-inhibitor iproniazid does not affect dextran oedema, contrary to expectation [36, 846, 1092];

that oedema produced by 5-HT, even after the highest doses which can be tolerated, is never as marked as that obtained with substances capable of producing an anaphylactoid inflammation;

that oedema produced by histamine, dextran or formaldehyde is inhibited to a great extent by pretreatment with glucagon (1 mg/kg), whereas 5-HT oedema is not significantly changed [713b];

that 48/80 even given repeatedly does not produce any change in the urinary excretion of 5-HIAA and rats given compound 48/80 in increasing doses for 8 days excrete as much 5-HIAA after injection of reserpine as the untreated controls do [618b];

finally, that the protection afforded by phenothiazine derivatives [909, 1177] towards anaphylactoid reaction does not parallel their anti-5-HT action [679].

Dextran does not seem to produce exactly the same effects as ovomucoid or egg-white [512, 712, 846]. The oedema caused by these agents, in turn, differs profoundly from that provoked by local application of formaldehyde [908]. No 5-HT mediation is suggested for formaldehyde oedema [1176].

9.3 Inflammation

Owing to the striking effect of intradermal or subcutaneous 5-HT on the calibre and permeability of capillaries in the rat skin, it has been repeatedly suggested that 5-HT may participate in acute vascular reaction to injury [81].

9.31 Cutaneous burns

Anaesthetized rats and mice were immersed in hot water at 60° C for 10 seconds. No changes in the 5-HT content of the skin, spleen or lungs could be observed at any time after the burn [48, 49]. Similarly, BOL causes only a slight and inconstant delay in the onset of the increased capillary permeability seen after thermal injury [1153, 1313c].

The output of 5-HIAA in rat urine presents a conspicuous drop immediately after burn injury (from 24 to less than 5 µg/rat/day) and then rises by the fourth post-burn day to 73 µg. Cyproheptadine, a potent 5-HT antagonist, alters neither the mortality nor the 5-HIAA excretion of the burned rats [504d].

Results of studies on urinary excretion of 5-HIAA in human patients following burns are at complete variance. Whereas JOHANSSON [620] found in burned

patients a conspicuous increase in urinary 5-HIAA, up to 35 mg/day, GOLD-SMITH *et al.* [504b] observed, in 10 burned patients followed for periods of 4 to 117 days, a persistently low 5-HIAA excretion (often less than 1 mg/day) from shortly after the burn until convalescence ensued.

In rabbits the only effect of thermal injury is to decrease the 5-HT concentration in whole blood as a consequence of a decrease in the platelet count [620].

9.32 Acute turpentine pleurisy in rats

Pleural exsudates were produced by injections of turpentine into the pleural cavity of rats. Thirty minutes after turpentine administration they contain remarkable amounts (1–3 $\mu\text{g/ml}$) of 5-HT, and 5-HT activity (1 $\mu\text{g/ml}$) has been found to develop in platelet-free exsudate from resolving pleurisy incubated with isolated liver mitochondria [1151]. On the basis of these data, SPECTOR and WILLOUGHBY suggested at first that 5-HT might be an important mediator of increased capillary permeability in the earliest phases of acute inflammation. However, later on, they abandoned this idea, because both BOL and reserpine pretreatment failed to influence accumulation of exsudate, and iproniazid and other MAO-inhibitors reduced, instead of further augmenting, the increased capillary permeability [1152, 1154].

9.33 Bacterial inflammation of the rat peritoneal cavity

Lethal infections were induced in the rat by *Ps. pyocyanea* and *C. ovis*, and lethal intoxication by *Staphylococcus aureus* α -toxin. Only negligible amounts of 5-HT and histamine were found in the peritoneal fluid and their release appeared to be epiphenomenal. It was concluded that the rôle of the two amines in the early reaction to the acute infection is not essential [1142].

9.34 Vascular responses to *E. coli* endotoxin

Intravenous injections of a heat-killed suspension of *E. coli* into a rabbit provoke a febrile reaction accompanied by two phases of constriction in the vessels of the denervated ear, one early and the other delayed.

Both phases are due to the appearance of filtrable substances in the circulating blood. The substance in the early phase resembles 5-HT (up to 10–20 $\mu\text{g/litre}$), those in the delayed phase resemble adrenaline and 5-HT (0.5–1 $\mu\text{g/litre}$) [34, 33]. Similarly, the fact that pretreatment with Dibenamine® and pyrilamine, Dibenamine® alone, or LSD with or without Dibenamine®, diminishes or prevents the acute hypotensive response of the cat to small doses of endotoxin is considered by GILBERT [490] to be compatible with the thesis that histamine and 5-HT are released and play a rôle in the early stages of the endotoxin reaction in the cat.

9.35 Protective action of 5-HT towards infections and bacterial toxins

Depletion of histamine by repeated injections of polymyxin B, or of 5-HT by injections of reserpine causes in the rat a breakdown of the resistance against infection by *Staph. aureus* [835]. On the other hand, 1 mg/kg 5-HT, given

subcutaneously, affords significant protection to adult mice against intraperitoneal injections of purified *E. coli* endotoxin [505].

9.36 Local Schwartzman reaction

5-HT injected intravenously or locally into the abdominal skin of rabbits treated with an intradermal injection of an active polysaccharide prepared from *Serratia marcescens* does not, unlike catecholamines and Pitressin®, provoke any local reaction [970].

9.4 Reaction to stress

According to WEST [1299], 5-HT plays a major rôle in the rat in the reaction of the organism to the stress and strain of life, particularly with respect to its location in the skin, in areas which are in contact with the outside world. In fact, 5-HT increases in concentration under physiological conditions involving a fresh environment (two days after weaning the 5-HT values of the skin are about double those found in unweaned rats of the same age) [902] or an increased use of a tissue (the tongue of females has more than twice the amount of 5-HT found in the tongue of males) [906].

Similarly, blood 5-HT concentration increases in adrenalectomized rats subjected to shock by limb ischaemia obtained with a rubber tourniquet [816].

The above opinion of WEST does not seem to be invalidated by experimental results demonstrating that large doses of exogenous 5-HT lower the ability of intact or adrenalectomized rats to withstand cold stress [1352b], and do not affect but even increase the mortality of rats exposed to drum shock [808]. It is possible, in fact, that decreased resistance of animals to cold and trauma is due simply to the unfavourable effect of exogenous 5-HT on blood pressure and body temperature. In man muscular effort during athletics, even when accompanied by considerable nervous excitement, did not produce any change in the urinary excretion of 5-HIAA [1084b].

10. Blood 5-HT and Urinary 5-HIAA Excretion in Human Morbid Syndromes

10.1 Carcinoidosis

This morbid syndrome, due in its most typical clinical manifestations to excess production of 5-HT by a malignant metastatic argentaffinoma, has been thoroughly investigated by numerous research workers and has been the object of excellent review articles [970b, 985b, 565b, 1145b, 1197b, 1125, 1274b, 1149b]. The reader is referred to these articles for complete information on the topic.

It will be seen, however, that biochemical results obtained in the studies on carcinoidosis have been repeatedly quoted and discussed in this paper also.

10.2 Symptomatic hyperserotoninemia without carcinoidosis

PICHEL WARNER [920b] has recently described a new morbid syndrome which is characterized biochemically by increased blood 5-HT levels with subnormal urinary 5-HIAA levels and with subnormal urinary recovery of 5-HIAA following oral administration of 5-HT, and clinically by abdominal pain, nausea and vomiting, diarrhoea, weight loss, weakness and easy fatigability, increased gastric secretion on x-ray examination, and possibly psychiatric disturbance. All the patients affected by the syndrome had recurrent episodic attacks, but most or all had their complaints to a lesser intensity between attacks. A provocative test of intramuscularly administered reserpine produced a severe typical attack of abrupt onset. Therapy with cyproheptadine produced distinct improvement in those patients of the group able to tolerate the drug.

PICHEL WARNER suggests that the syndrome has at its root an aberration of 5-HT metabolism, possibly in association with diminished monoamine oxidase activity.

10.3 Blood diseases

The 5-HT content of whole blood, platelet-rich plasma and serum has been estimated in numerous blood diseases by HARDISTY *et al.* [545, 547] and by SCHMID *et al.* [1080].

The main results are summarized in Table 14.

SHULMAN *et al.* [1116] have found in von Willebrand's disease a normal 5-HT content in blood and platelets and a normal urinary excretion of 5-HIAA; in haemorrhagic thrombocytaemia a normal blood 5-HT concentration and urinary excretion of 5-HIAA but a content of 5-HT per platelet which is only 10% of the normal; and, finally, in thromboasthenia a normal blood 5-HT and urinary 5-HIAA excretion with a 5-HT content per platelet above normal.

POLLI *et al.* [951] have observed that in their cases of leukaemia the 5-HT content per 10^9 platelets is always reduced: from 1.65 to 0.4 μg in myeloid leukaemia and to 0.17 μg in non-differentiated leukaemia.

Finally, ZUCKER and BORELLI [1356] found very low 5-HT levels in 3 cases of thrombocytopathia, but in 3 other cases normal levels; in pseudo-haemophilia normal levels; in thrombocytosis normal levels (in 6 out of 9 patients), subnormal levels (1/9) or levels above normal (2/9).

All the above investigators unanimously agree that platelet 5-HT and the platelet count are unrelated to each other, that there is no reason to attribute the bleeding in haemorrhagic blood diseases to 5-HT deficiency, and that 5-HT levels in blood and platelets and 5-HIAA excretion in urine are not related to platelet function in haemostasis.

10.4 Diseases of the mast-cell system

Tissues from the liver and spleen of a child afflicted by *urticaria pigmentosa* [458] and of another subject suffering from mastocytosis of the spleen [358]

Table 14
The 5-HT content of blood in blood diseases

	Whole blood 5-HT ($\mu\text{g/ml}$)	Platelet 5-HT (10^9)	Serum 5-HT ($\mu\text{g/ml}$)
Normal subjects	0.16	0.57	0.12
Myeloid leukaemia { acute	0.116	0.24	0.006
chronic			0.3
Lymphatic leukaemia	0.09	0.28	0.06
Pernicious anaemia	0.06	0.20	0.013
Polycythaemia vera	0.07	0.18	0.047
Myelofibrosis	0.015	0.14	=
Haemorrhagic thrombocytaemia	0.24	0.26	=
Hodgkin's disease	0.16	0.31	0.07
Iron-deficiency anaemia	0.10	0.33	=
Thrombocytopoenic purpura	0.007	0.22	0.04
Haemophilia	=	=	0.04
Abdominal carcinomatosis	0.05	0.2	=
Acute reticulosis	=	=	0.003
Chronic reticulosis	=	=	0.128
Multiple myeloma	=	=	0.096
Medullary carcinosis	=	=	0.034

did not show any content of 5-HT in spite of conspicuous infiltration with mast cells. Similar findings have been obtained on examination of skin specimens from other cases of *urticaria pigmentosa* (even urinary 5-HIAA excretion was found to be normal) [1126] and of a human mast cell tumour [906].

The above negative results are in accordance with results obtained in cases of mastocytoma of cattle and dogs, but differ sharply from the positive results obtained in cases of mice mastocytoma (mean 5-HT content of the tumoral tissue 326 $\mu\text{g/g}$, range 6–714 $\mu\text{g/g}$) and of mice mast-cell leukaemia (7.74 μg 5-HT per ml of platelet-rich plasma, instead of the normal 2.2 μg [872, 518]).

10.5 Cardiovascular diseases

Hypertensive disease. Generally, patients with essential hypertension excrete normal amounts of 5-HIAA in their urine [553, 307] and only a minor number of subjects exhibit an urinary 5-HIAA level below the normal [146].

Neurocirculatory asthenia. The urinary excretion of 5-HIAA proved to be identical in 29 control subjects and in 39 patients with neurocirculatory asthenia [864].

Acute coronary failure. The statement of ANGELINO *et al.* [17b] that patients suffering from acute coronary failure show an increased urinary excretion of 5-HIAA awaits confirmation, owing to incompleteness of reported data (urine volume, for example, is lacking).

Pulmonary thromboembolism. It has been suggested by SMITH and SMITH [1141], on the basis of experimental work carried out in cats, that the signs and symptoms of pulmonary embolism might depend on the release to some considerable extent of 5-HT from both the blood clot and the infarcted area of the lung.

However, experimental results obtained in man, dogs [1036] and rats [442] could not corroborate the above theory and it is now generally affirmed that the role of 5-HT in the clinical picture of pulmonary thromboembolism in man is probably negligible [1036, 308b]. This negative conclusion is based above all on the fact that it is unlikely that the very minute amounts of 5-HT contained in human blood, clots of human blood and lung tissue could provoke any pharmacological effect on the lung vessels, and on the observation that the injection of 5-HT (4–400 μ g base) through the distal lumen of a catheter inserted into the pulmonary artery before and after unilateral balloon occlusion of the corresponding artery produces no objective or subjective changes resembling those associated with pulmonary embolism [1178d].

Raynaud's disease and Raynaud's phenomenon. HALPERN *et al.* [540] have observed that changes in the calibre of the finger vessels and in the digital temperature are similar, in many respects, during the cold-stressor test in patients suffering from Raynaud's disease and during the infusion of 6 μ g 5-HT base per minute, for 10 minutes into the brachial artery of normal subjects.

On the basis of this and other observations, these investigators have postulated an integrated explanation for the mechanism of Raynaud's phenomenon, as follows: 'On exposure of the hands to cold, a peripheral vasoconstriction occurs as a result of both sympathetic reflexes and local response to physical stress. The vasoconstriction gives rise to blanching, and, by obstruction to flow in the small vessels, results in a destruction of some of the blood platelets, thereby releasing 5-HT. Through the local action of 5-HT the vasospasm originally induced by the sympathetic vasoconstriction forces is reversed and the epinephrine dilated arterioles are constricted, while the capillary bed is dilated. This virtual obliteration of the smaller vessels with a dilated capillary bed results in hemostasis and cyanosis. Simultaneously local failure of the MAO system permits the accumulation of vasoactive amines which ultimately, through tachyphylaxis or an antagonist effect, reverse the pattern of vasoconstriction and vasodilatation. With the resumption of the equilibrium of vascular tone the local excesses are removed and the symptom-complex is abated.'

Although nobody at present can exclude the possibility that 5-HT plays a rôle in controlling vasomotor tone and hence in the pathogenesis of peripheral vascular diseases, it is a fact that the above tentative explanation of Raynaud's phenomenon lacks any sufficiently solid biochemical and pharmacological basis. It is, for example, surprising that no platelet count and estimation of 5-HT concentration has been carried out during Raynaud's phenomenon on the blood returning from the hand exposed to cold and for comparison, in the blood returning from normal vascular areas.

10.6 Diseases of the liver and gastro-intestinal tract

No significant changes in blood 5-HT levels and/or in urinary excretion of 5-HIAA could be demonstrated in patients with gastric or duodenal ulcer [1081, 1077, 655], gastric carcinoma [1081], gastric achlorhydria [182], diarrhoea due to irritable colon, ulcerative colitis, regional enteritis and pancreatic disease [1081, 678], nor in patients recently operated for partial resection of the stomach [1081].

Blood 5-HT levels are also normal in patients suffering from acute liver disease, including biliary tract obstruction, but subnormal in patients afflicted by liver cirrhosis [1081, 364].

However, even in these patients basal daily excretion of 5-HIAA and percentage recovery of 5-HIAA after administration of 5-HT and 5-HIAA do not differ from the normal [212, 334, 335b, 335c].

The only doubtful sign of hepatic decompensation (not confirmed by BORGES *et al.* [147]) is a significantly greater recovery of administered 5-HTP as 5-HIAA (62% instead of 32%), possibly due to impairment of 5-HT conjugation in hepatic cirrhosis [334, 335b].

Partial resection of the stomach and small intestine has little effect on blood 5-HT levels and urinary 5-HIAA [1081], but total resection of the colon and [97], much more so, resection of the entire small bowel and part of the large intestine [995] produces a significant or even tremendous decrease in both serum 5-HT and urinary 5-HIAA.

In patients suffering from symptomatic non-tropical sprue urinary 5-HIAA is considerably increased above normal (11–16 mg instead of the usual 2–8 mg/24 hours) but values return to normal during remission associated with elimination of gluten and gliadin from the diet. It is possible that the increased excretion of 5-HIAA in active non-tropical sprue reflects a defect in the metabolism of tryptophan [678].

Intestinal sterilization with oxytetracycline has no effect on 5-HIAA excretion [182].

10.7 Miscellaneous diseases

Rheumatoid arthritis. Whereas the blood 5-HT content in rheumatic fever is approximately normal [1078, 1079, 1264b], it is significantly decreased in rheumatoid arthritis (from 0.12 to 0.034 μg [1078, 1079]), and from 0.16 to 0.10 $\mu\text{g/ml}$ [646]).

Similarly, the concentration of 5-hydroxyindoles in morning urine is slightly lower in arthritic patients than in controls [869].

Moreover, the absorption of 5-HT by thrombocytes from patients with rheumatoid arthritis and with non-rheumatoid inflammatory states is significantly decreased as compared to individuals with no demonstrable inflammation [647].

Finally, patients with active rheumatoid arthritis or systemic *lupus erythematosus* demonstrate an exaggerated response to 5-HT (intradermal, peri-articular and intraarticular injections) characterized by rapid spread of ery-

thema, swelling and cyanosis, lasting from 1 to 8 hours. Control subjects show only mild localized erythematous swelling without cyanosis [1065, 1066].

The exaggerated response to extravascular injection of 0.1 mg 5-HT in patients with rheumatoid arthritis is antagonized by i.v. administration of 2 mg BOL or 1-methyl-methergine [1067c].

Allergy. Significant differences have been found by MAC HAFFIE [761] in allergic human beings, in the direction of increased serum 5-HT levels (from 0.15 to 0.20 $\mu\text{g/ml}$). These differences are not changed by allergic hyposensitization with appropriate antigens.

Bilateral renal disease. 5-HIAA excretion is increased in 50% of the patients suffering from bilateral renal disease. The increase is independent of the nature of the disease and of the behaviour of blood pressure [11].

Chronic alcoholism. The daily basal excretion of 5-HIAA has been found to be significantly lower in 34 patients with chronic alcoholism (2.5 ± 1.0 mg) than in 16 normal control subjects (6.4 ± 1.4 mg). After a tryptophan load, the patients with chronic alcoholism excrete again less 5-HIAA (5.2 mg/24 hr) than control subjects (11.6 mg/24 hr). A study of 4 patients for periods up to four months indicates that the low level of 5-HIAA is not changed by improved nutritional status or abstinence from alcohol. Since the kynurenine pathway is normal in the chronic alcoholic, it is suggested that in chronic alcoholism there is a preferential depression in the conversion of tryptophan to 5-HIAA [871b].

In the writer's opinion the available data point to a defect in the 5-hydroxylation of L-tryptophan by the intestinal mucosa rather than to a defect in decarboxylation of 5-HTP or in deamination of 5-HT.

Single moderate doses of ethanol (0.5–0.7 g/kg) ingested by healthy young subjects do not produce any change in the urinary excretion of 5-HIAA and histamine. This makes it unlikely that flushing caused by moderate ethanol doses is associated with the release of 5-HT or histamine [915b].

Toxaemia of pregnancy. Abortion. It has been suggested by some investigators that 5-HT may play a role in toxaemia of pregnancy [685, 888] and in abortion [1244], but this suggestion is rejected decidedly by others [1055, 24] mainly on the basis of lack of any sign of altered metabolism of 5-HT in these morbid syndromes.

Similarly, 5-HT does not seem to play any part in the menopausal flush. In fact, 5-HIAA excretion is not increased in the menopausal syndrome and estrogen inhibition of flushes does not affect 5-HIAA output in urine [852, 1009b].

In cases of gynaecological tumours, excretion of 5-HIAA is within normal limits [1055].

Data on urinary excretion of 5-HIAA during normal pregnancy are at complete variance. BETTENDORF *et al.* [106] find a sharp reduction of urinary 5-HIAA (volume of urine not given!) in the second half of pregnancy with return to normal values soon after delivery; SAURAMO *et al.* [1055], and PIGEAUD *et al.* [931b] on the contrary, observe a moderate increase in the daily output of 5-HIAA (10–15 mg/day) during the 2nd and 3rd trimester of pregnancy, and

a fall at the moment of delivery. ISRAEL *et al.* [613b] could not find any relationship of 5-HT to labor. In fact, neither blood contains more 5-HT nor urine more 5-HIAA during labor.

It should be added that in the rat, urinary 5-HIAA excretion remains unchanged during pregnancy [24].

No changes in the average urinary excretion of tryptamine (normal 36–120 $\mu\text{g/day}$) have been found in patients with essential hypertension, pheochromocytoma, coronary heart disease, intestinal obstruction with sprue, asthma or epilepsy [1129].

11. Drugs which Antagonize 5-HT

5-HT antagonists or anti-5-HT drugs are compounds capable of preventing or blocking, both *in vitro* and *in vivo*, the pharmacological effects of 5-HT. They do not interfere with biosynthesis, release or metabolism of endogenous indolealkylamines.

It is evident that 5-HT antagonists may be of interest from several points of view:

a) They may greatly help in the distinction, especially in crude tissue extracts, of 5-HT from other active tissue products.

b) They may substantially contribute to our understanding of the mechanism of action of 5-HT as well as of other drugs supposed to act through 5-HT liberation.

c) Finally, they may be of importance in the prevention or treatment, in human beings, of some side effects produced by exogenous indolealkylamines or precursor aminoacids, and in the diagnosis and treatment of morbid syndromes attributable to excess 5-HT production or to anomalous sensitivity of receptors towards circulating 5-HT.

Of course, desirable qualities of 5-HT antagonists would be low toxicity, high potency and high specificity of action, together with a broad spectrum of activity on the 5-HT receptors in the most different organs and tissues.

Although some of the available 5-HT antagonists fulfil many of the above requirements, none completely does so, and none has given in man therapeutic benefits which could be attributed with certainty to its anti-5-HT action.

11.1 Analogues of 5-HT and other indole compounds

11.11 Tryptamines

Excess 5-HT or tryptamine is capable, when left in contact with the isolated smooth muscle preparation, of abolishing the effect of subsequent doses of 5-HT [448b].

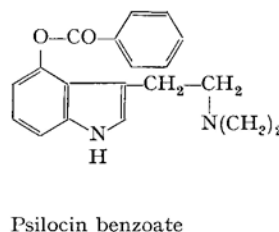
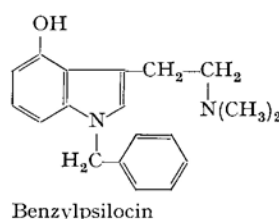
Moreover, 5-HT given by the parenteral route protects the guinea-pig against bronchospasm produced by 5-HT aerosols, while leaving unaffected the asphyxial coma caused by histamine and acetylcholine [282].

A number of N-alkylated derivatives of 5-HT and tryptamine have been studied by BERTACCINI and ZAMBONI [100] and have been found to produce a reversible, competitive blocking of the spasmogenic actions of 5-HT on smooth muscle preparations. It is noteworthy that the antagonistic action is regularly more intense on the guinea-pig ileum than on the rat uterus.

The most interesting of these compounds are: N,N-dimethyltryptamine, N,N-diethyltryptamine, N,N-dipropyltryptamine, N,N-dibutyltryptamine, N,N-dibutyl-5-methoxytryptamine, N,N-dibutyl-5-HT, 5-methoxy- β -methyltryptamine and β -hydroxy-bufotenine.

Starting from the observation that psilocin and also psilocybin, like bufotenine and N,N-dimethyltryptamine, show definite antagonistic action towards 5-HT, CERLETTI *et al.* [249] succeeded in preparing psilocin derivatives which manifest anti-5-HT action considerably greater than that possessed by the parent substance.

1-Benzylpsilocin and psilocin benzoate, for example, have 10 and 18%, respectively, of the antagonistic potency of LSD.



11.12 Methyl-derivatives and benzyl analogues of 5-HT and their ethers

These compounds show anti-5-HT activity not only on isolated artery rings and isolated uteri, but also on the blood pressure of dogs and other experimental animals [1322, 1319, 1093, 1094, 1096], as well as on the diarrhoea produced in mice by 5-HTP [1330].

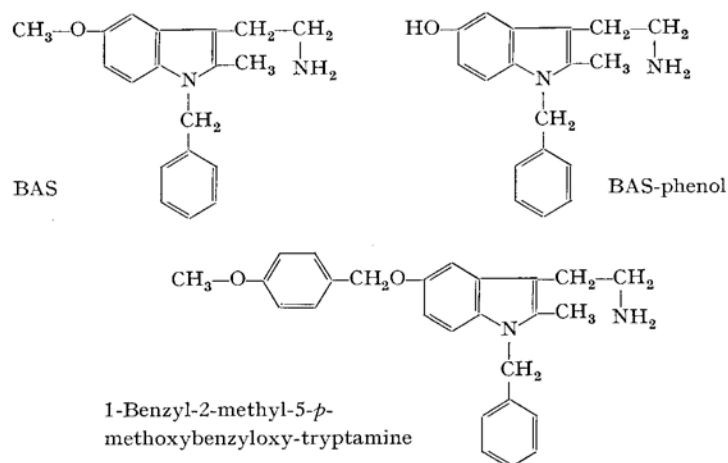
The most active members of this series are listed below with their inhibition index, i.e. the number of molecules of the antimetabolite necessary to produce a 50% inhibition of the contraction of the rat uterus produced by one molecule of 5-HT, when a dose of 5-HT capable of causing a maximum contraction is used.

2-methyl-5-HT	1000
2-methyl-5-methoxytryptamine	300
1, 2-dimethyl-5-methoxytryptamine	200
1-methyl-5-methoxy-N, N-dimethyltryptamine	300
1-benzyl-5-methoxytryptamine	40
1-benzyl-2-methyl-5-methoxytryptamine (BAS = benzylanalogue of serotonin)	30
1-benzyl-2-methyl-5-HT (BAS-phenol)	=

1-benzyl-2-methyl-5-methoxy-N,N-dimethyltryptamine (BAB = benzylanalogue of bufotenine)	15 [1093]
1-benzyl-2-methyl-5-(<i>p</i> -methoxybenzyloxy)tryptamine	8
1-benzyl-2-methyl-5-(oxycacethydrazide)tryptamine	3 [1330]

When given by the oral route BAS, BAB and BAS-phenol are approximately of the same potency in protecting dogs against the pressor effects of 5-HT, the dose of the antimetabolite required for preventing the effect of 40 to 60 $\mu\text{g/kg}$ 5-HT being 1 mg/kg/day, for several days.

Intravenously, BAS-phenol is about 10 times more active than BAS (350 $\mu\text{g/kg}$ antimetabolite against 50 μg 5-HT); BAB is less active than BAS-phenol, but more active than BAS. Both BAS and BAB are feeble antagonists of 5-HT on the isolated guinea-pig lung [1180]. Similarly, BAS poorly antagonizes the pressor effect of 5-HT in the rat, while sometimes increasing the depressor effect [875].



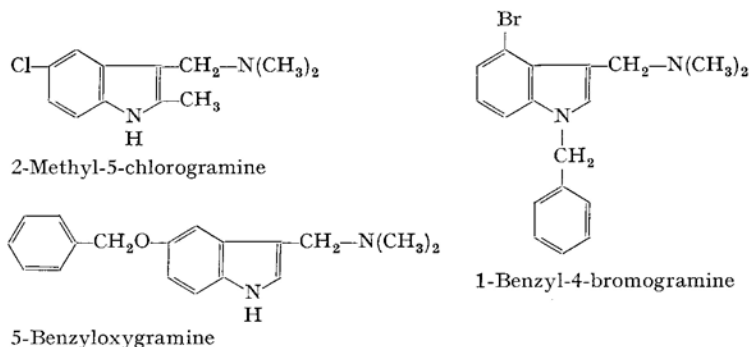
Surprisingly enough, BAS-phenol is completely ineffective in the dog towards the pressor effects of tryptamine, thus suggesting the existence in the vessels of separate receptors for 5-HT and tryptamine [1325].

11.13 Gramines

Gramine and several 2-, 5- or 4- substituted gramines possess a remarkable anti-5-HT action *in vitro* (rat uterus, guinea-pig ileum, perfused rabbit ear), but only a moderate and short-lasting action *in vivo* (dog blood pressure, dog urinary bladder, rat antidiuresis).

The inhibition index for some compounds of this series is as follows, keeping in mind that the figures below are merely indicative, owing to the different methods and criteria used by the various investigators in evaluating anti-5-HT potency:

Gramine	30-60	1-Benzyl-4-bromogramine	1 [249]
2-Methyl-5-chlorogramine	3- 4	4-Cyanogramine	50
2-Methyl-5-bromogramine	2- 4 [376]	4-Carbomethoxygramine	15 [550]
5-Benzyloxygramine	10 [448]		



11.14 4-Substituted indoles

Besides the above described 4-substituted gramines, several other 4-substituted indoles have been found to antagonize the action of 5-HT on the rat uterus preparation.

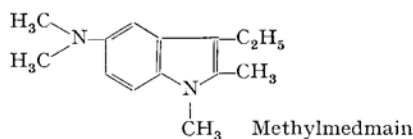
In parentheses the inhibition indexes of the best are given: 4,4'-dicyano-3,3'-diindolylmethane(5), 4-indolecarboxylic acid diethylamide(50), and 3,3'-diindolyl-methane(20) [550].

11.15 Indole carboxamidines

These compounds display a moderate anti-5-HT action on the rat uterus preparation (optimum inhibition index=25-50), the isolated carotid strip and the dog blood pressure, when given intravenously. They are ineffective by the oral route [1097].

11.16 Aminoindoles and nitroindoles

These antimetabolites of 5-HT, the first one synthesized by WOOLLEY and SHAW [1318b] have now lost their original interest almost entirely. The main representatives of the group are 2-methyl-3-ethyl-5-aminoindole, 2,3-dimethyl-5-aminoindole, 2-methyl-3-ethyl-5-dimethyl-aminoindole (*medmain*) and 1,2-dimethyl-3-ethyl-5-dimethyl-aminoindole (*methylmedmain*).



11.17 Substituted β -aminoethylindazoles

The benzyloxyderivatives of the indazole analogues of 5-HT and bufotenine possess a moderate anti-5-HT action on the rat uterus, and a more intense action on the guinea-pig ileum [9b].

11.2 *D*-Lysergic acid diethylamide (LSD) and related compounds

This group includes the most potent antagonists of 5-HT so far known.

The powerful antagonistic action of LSD towards 5-HT was first discovered by GADDUM in 1953 [454, 447b] and soon confirmed by numerous research workers. It may be said that the majority of the peripheral actions of 5-HT and related indolealkylamines are prevented or abolished by LSD. Antagonism, which is typically competitive [448], is prompt and generally long-lasting or even unsurmountable.

The stimulant action of 5-HT on the isolated rat uterus [447b, 448] and the constrictor action on the perfused rabbit ear [1052] are blocked by doses of LSD as low as 0.5–1 μ g per litre nutrient liquid, and parenteral doses of LSD as low 10–50 μ g or 2–10 μ g/kg are sufficient to antagonize the bronchoconstrictor action of 5-HT in the guinea-pig [569, 616] and the antidiuretic action in the rat [364], respectively.

It has been affirmed by DELAY and THUILLIER [310] that very low doses of LSD may potentiate the action of 5-HT on the rat uterus, but this has not been confirmed by others [247].

LSD is not a pharmacologically inert drug. Apart from its tremendous central effects, it displays evident peripheral actions, which in some cases may be decidedly 5-HT-like, thus more or less completely masking eventual concomitant anti-5-HT effects. This is especially true in the heart of some molluscs, on which LSD is a more powerful stimulant agent than 5-HT itself [1095].

LSD was the first representative of a formidable group of compounds, which have been developed and studied at the SANDOZ Laboratories in Basle [246, 248, 245, 249, 247, 330, 1006, 1007, 1212, 501]. The most important of them are listed in Table 15, in which the anti-5-HT potency of LSD on the rat uterus preparation is arbitrarily considered as 100, and that of the other compounds expressed in percentages.

Comparative studies have demonstrated clearly that the relative potency of the above lysergic acid derivatives varies greatly according to the test object.

MLD, for example, is 200 times more potent than LSD in inhibiting the 5-HT-induced barbiturate potentiation in mice [247]; BOL is considerably more potent than LSD against the spasmogenic effect of 5-HT on the rabbit and guinea-pig intestine [1147]; 1-methyl-LSD and 1-methyl-BOL, do not surpass LSD or BOL in counteracting oedema formation caused by 5-HT in the rat paw [330].

Whereas the anti-5-HT effect of BOL and UML, as determined *in vitro* on the rat uterus, removed at various time-intervals after the injection of the

alkylamines and not only for 5-HT, it should be stressed that our present knowledge of the active compounds which may be found in tissues is too limited to allow us to exclude the possibility that other substances, besides indole-alkylamines, are antagonized by LSD. It is evident that these reservations apply to all other antagonists of 5-HT.

BOL, 2-methyl-N,N-dimethyltryptamine and 5-benzyloxy-N,N-dimethyltryptamine antagonize the effects of 5-HT on the rat fundus strip more than those of tryptamine. It seems that the differential character of the blocking action of these compounds should be ascribed either to interferences with the transport of tryptamine through the cell wall or to the existence of separate tryptamine and 5-HT receptors [59b].

11.3 Chlorpromazine and related phenothiazines

The anti-5-HT action of chlorpromazine was first described by GYERMECK [526, 527] and then confirmed by other research workers.

Chlorpromazine has been found to antagonize the effects of 5-HT on the rat uterus (threshold concentration 2×10^{-9}) [527, 83], the rat colon (10^{-6} – 10^{-7}) [83, 616], the guinea-pig ileum (10^{-7}) [616], the capillaries of the rat paw (1 – $10 \mu\text{g/kg}$) [83, 616, 330], the blood pressure of decapitated cats or of cats under ganglionic blockade (1 mg/kg) [526, 527] and, finally, the effects of 5-HT on the guinea-pig lung preparation (good effect when bronchospasm is incomplete, little effect when complete [652]) and on the bronchial musculature of the intact guinea-pig (5 – 10 mg/kg) [80, 568].

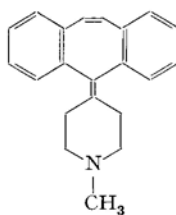
The other phenothiazine derivatives examined are, with a few limited exceptions, less potent than chlorpromazine. Of interest, but not proved, is the suggestion that the anti-5-HT activity of the phenothiazines might parallel their sedative action [282].

The blocking effect of chlorpromazine towards 5-HT, while sometimes very remarkable, especially *in vitro*, does not appear to be specific. In fact, the drug antagonizes the bronchoconstrictor action of histamine, acetylcholine, methacholine and nicotine [568, 1074] and the intestine-stimulant action of acetylcholine as well as that of 5-HT [83]. Moreover, there is great variability in the 5-HT blocking capacity of chlorpromazine and related compounds not only depending on the different tests, but also on different preparations of the same test [527].

11.4 Antihistaminic drugs

The strongest anti-5-HT activity, among the antihistaminic drugs, is certainly possessed by cyproheptadine (1-methyl-4-(5-dibenzo-[a, e]-cycloheptatrienylidene)-piperidine), which is capable of blocking the action of 5-HT on the dog blood pressure, the spasmogenic effect of 5-HT on the isolated rat uterus (ED 50 for cyproheptadine $0.34 \mu\text{g/l}$, for LSD $22 \mu\text{g/l}$), and the swelling and oedema produced by local injections of 5-HT into the rat paw (ED 50 for cyproheptadine $30 \mu\text{g/kg}$, for LSD $20 \mu\text{g/kg}$, and for chlorpromazine 3 mg/kg).

An unusual characteristic of the antagonistic effect of cyproheptadine on the spasmogenic action of 5-HT on the rat uterus preparation is that whereas little or no inhibition occurs when the drug is present, along with 5-HT, in the bath, a powerful 5-HT block makes its appearance after cyproheptadine has been washed off [1178b].



Cyproheptadine

With the exception of cyproheptadine, recent research [652, 818, 1074, 330] has added but little to what has been known for a long time about the anti-5-HT activity of histaminolytic drugs.

It has been confirmed that there is no evident relationship between the anti-5-HT potency of these compounds and their histaminolytic, miolytic or local anaesthetic properties.

11.5 Sympatholytic drugs

Owing to the biochemical and pharmacological resemblances between 5-HT and catecholamines, sympatholytic drugs were among the first compounds successfully tried as antagonists of 5-HT.

Dibenamine®, *Dibenzyline*®, and the *polypeptide alkaloids of ergot* (natural and hydrogenated) are still listed among our best anti-5-HT drugs, especially *in vitro*. They provoke a prompt and powerful block of the 5-HT actions, which often is irreversible and unsurmountable.

Dibenamine® and *Dibenzyline*® seem to be the most suitable agents of this group. In fact, 0.1–0.5 µg/ml *Dibenamine*® is sufficient to counteract the effect on the rat uterus of even enormous concentrations of 5-HT, and *in vivo* the anti-5-HT action of the drug could be demonstrated, like its sympatholytic action, for as long as 8–10 days [364].

While 5-HT antagonism of sympatholytic drugs is of satisfactory specificity on the rat uterus, the same, of course, may not be true for other isolated preparations and for the intact animal [446].

11.6 Miscellaneous antagonists

Sympathomimetic amines. As expected, a number of sympathomimetic amines are capable of antagonizing the spasmogenic effects of 5-HT on various isolated preparations of the intestine and uterus, and on the bronchial musculature of the guinea-pig. By far the most potent is isopropylnoradrenaline,

active on the rat colon and on the guinea-pig ileum in concentrations as low as 10^{-9} and $2 \cdot 10^{-10}$, respectively [616].

Adrenochrome. According to STERN *et al.* [1178], 5–10 $\mu\text{g/ml}$ of freshly prepared adrenochrome is capable of blocking the spasmogenic effect of 0.04 to 0.08 $\mu\text{g/ml}$ 5-HT on the guinea-pig ileum.

Heparin and related drugs. The anti-5-HT potency of heparin and other anticoagulant drugs is very low, and, in addition, non specific. 2.5–5 mg heparin are required to block the action of 0.02–0.1 μg 5-HT on the rat uterus or rat colon [643, 1141]. Phenprocoumon is effective at doses of 50–100 $\mu\text{g/ml}$ and acenocoumarin at doses of 10–20 $\mu\text{g/ml}$ [1075]. *In vivo* a moderate protective action against the bronchoconstrictor effect of 5-HT in guinea-pigs and against the hypotensive effect of 5-HT in cats [1141] has been described.

Practically, the *in vitro* anti-5-HT activity of these drugs may be of some interest only if they are present in blood or serum subjected to bioassay for 5-HT.

AUSTERN [38b] has reported a case of carcinoid tumour of the ileum in which heparin Vitrum, given intramuscularly or intravenously in daily doses of 400–500 mg, caused remarkable improvement of the morbid syndrome. It is suggested that heparin may depress increased 5-HT production.

γ -Aminobutyric acid. GABA is antagonistic to 5-HT on the isolated guinea-pig ileum, but lacks any antagonistic property on the rat uterus and other smooth muscle preparations [607].

Fatty acids. N(2-hydroxyethyl) palmitamide ethanolamine HCl obtained from egg yolk, peanut oil and soya bean lecithin, and other fatty acids are found to diminish, in doses of 5 mg/kg, the 5-HT toxicity in mice sensitized with *B. pertussis* [457].

Tetrazotized diorthoanisidine. The tetrazo compound (10–40 mg/kg) reduces or abolishes the depressor response to 5-HT in rabbits, but does not alter the pyretogenic effect of combined treatment with isoniazid and 5-HTP [234].

Extracts of Rumex obtusifolius. The extract corresponding to 0.5 mg dock leaf reduces, with all smooth muscles used, by more than half the response to 5 μg 5-HT. This observation is of interest because dock leaves rubbed on the human skin are said to counteract pain due to nettle stings, containing 5-HT [163].

Reserpine. Reserpine, in considerable concentrations (1–25 $\mu\text{g/ml}$) often reduces the stimulant action of 5-HT on the rat uterus, rat colon, guinea-pig ileum [492] and isolated strips of carotid arteries [654].

In the intact guinea-pig parenteral doses of reserpine (1–3 mg/kg) are either ineffective [80] or moderately effective [616] against bronchoconstriction produced by 5-HT aerosols. The antagonistic action is non specific.

5-HT antagonists of low potency and generally of poor specificity are papaverine [568], aminophylline [568], Trasentine® [616], pentolamine [616], morphine [454], hydrallazine [654], nicotinamide [1329], Ritalin® [616], N-methyl-N-propyl-tetrahydronaphthylamine [913] and bulbocapnine [1273b].

The following concluding remarks may be made concerning the problem of drugs antagonistic to 5-HT:

1) Several drugs are available at present which are capable of displaying a powerful antagonistic action towards the *in vitro* effects of 5-HT on isolated smooth muscle preparations. Owing to their potency and specificity of action, LSD, BOL and related lysergic acid derivatives occupy a pre-eminent position, and their usefulness in the differential diagnosis of 5-HT from other active tissue products is unrivalled.

However, satisfactory results may also be obtained with some sympatholytic drugs (Dibenamine®, Dibenzyline®), cyproheptadine, some ergamine derivatives, and the benzyl analogues of 5-HT.

2) Lysergic acid derivatives, cyproheptadine, Dibenamine®, BAS and BAS-phenol retain also good anti-5-HT activity *in vivo*. It should be stressed however that the *in vivo* actions of 5-HT on various organs and functions are counteracted by the antagonists to a strikingly different degree [1034].

3) Attempts to use 5-HT antagonists in man for therapeutic purposes have been substantially unsuccessful and the partially positive results cannot be correlated with reasonable certainty to any block of local or systemic excess of 5-HT.

5-HT antagonists have been tried in man

a) in carcinoidosis, the only morbid syndrome definitely attributable to excess of circulating 5-HT,

b) in hypertension, on the assumption that 5-HT interferes in the pathogenesis of this disease,

c) in migraine, and, finally

d) in combination with 5-HTP, in an attempt to block the peripheral effects of the amino acid, leaving unaffected the central effects [1326].

Results obtained in carcinoidosis have been completely negative with BOL and BAS, even administered in relatively high doses, and only partially positive with UML [700]. The only 5-HT antagonist which causes some important amelioration of symptoms is chlorpromazine [591], but the pharmacological effects of this drug are too complex to permit the conclusion that it acts through 5-HT blockade.

In the treatment of hypertension BAS gives very modest, partial results, again with no certainty that they are due to its 5-HT antagonizing activity [1321, 1316, 1317, 1318].

UML has been found by SICUTERI [1117, 1118] and by HEYCK [575] of considerable value in migraine and in Horton's histamine cephalalgia, especially in their prophylactic treatment. Because the drug is one of the most potent antagonists of 5-HT, the hypothesis has been advanced of the existence of a specific connection between 5-HT and vasomotor cephalgic attacks.

Available evidence is against rather than in favour of this view.

In fact, parenteral administration of 5-HT (5–10 mg) or 5-HTP (10–20 mg) not only fails to induce headache in patients subject to migraine, but rather abolishes spontaneous migraine headache and relieves reserpine-induced migraine [649].

It has recently been claimed that UML produces amelioration of symptoms of rheumatism when injected intra-articularly or *loco dolenti* [623].

In the writer's opinion this is a strong additional argument against the view that UML acts through its anti-5-HT action.

In fact, there is as yet absolutely no evidence that 5-HT may participate in the production or maintenance of any symptom of rheumatism.

12. Drugs which Release 5-HT

BRODIE *et al.* [932, 1107, 1110] first demonstrated that reserpine is capable of releasing 5-HT from its body depots. This fundamental observation has been confirmed and extended by all subsequent investigators.

The effects of reserpine on extracerebral 5-HT will be described and discussed here. The extremely important effects of the drug on brain 5-HT and the implications of brain 5-HT release will be discussed in detail elsewhere.

12.1 Effect of reserpine on the various extracerebral stores of 5-HT

Blood platelets. Blood platelet 5-HT is released by reserpine with exceptional ease both *in vitro* and *in vivo* and reserpine is capable, again both *in vitro* and *in vivo*, of hindering the absorption by the platelets of subsequent 5-HT. Thus, release of 5-HT from the thrombocytes has been thoroughly studied in order to clarify the mechanism of action of reserpine.

Following intravenous injections of 0.15–5 mg/kg reserpine the 5-HT content of rabbit blood declines progressively, reaching 50% of the normal after 4 hours and 2–5% after 16 hours. This low level persists for 2–3 days and then increases slowly, reaching a normal value after about 6 days. Single doses of reserpine as low as 0.015 mg/kg are sufficient in rabbits to decrease blood 5-HT by 75% [1109, 856].

Similar effects have been observed in all other examined animal species:

α) In rats 5 mg/kg reserpine, intraperitoneally, produces a rapid decline of blood 5-HT, in the order of 25–30% already after one hour, of 60–70% after 2 hours, and of 90–95% after 4–40 hours. Recovery is still incomplete after 6 days. Single doses of 0.1 mg/kg cause a 35% decline in blood 5-HT [366].

β) In pigeons maximum decrease, observed after 24–48 hours, is 95–98% with 5 mg/kg reserpine, but doses as low as 0.1 mg/kg are still effective. Recovery is complete after 5 days [377].

γ) In guinea-pigs reduction of platelet 5-HT after 5 mg/kg reserpine is 90% [1109].

δ) In man a single intramuscular injection of 1 mg reserpine is sufficient to remove virtually the whole of the 5-HT in the platelets (from 0.036 μg per 10^8 platelets to 0.001 μg after 3 days). In some subjects recovery is still incomplete after 21 days, suggesting that reserpine affects not only platelets in circulation but also platelet-bearing tissue [546].

Repeated administration of small doses of reserpine, 0.01–0.015 mg/kg for 7–14 days, produces both in man and experimental animals a cumulative

effect, and in all instances the blood 5-HT level never exceeds 5 to 10% of the normal.

It should be stressed immediately that this cumulative effect of small doses of reserpine is much less evident in the brain and still less in the intestines [552, 554, 379].

Blood platelets isolated from guinea-pigs or human beings 1–9 days after administration of reserpine show a marked, 75–90%, deficiency in their ability to take up 5-HT from the suspending medium [546, 682, 172].

Iproniazid inhibits the reserpine-induced 5-HT decrease in the platelets [1346]. Since the same effect is obtained by pretreatment of the animals with large doses of exogenous 5-HT (25 mg/kg. s.c.) [366], it is doubtful whether the effect of iproniazid is directly related to MAO inhibition, as suggested by PAASONEN and PLETSCHER [884].

As already stated, reserpine is a powerful liberator of platelet 5-HT *in vitro*, too. SHORE *et al.* [1108, 1113] found that very low concentrations of reserpine (minimum active concentration 0.05 $\mu\text{g/ml}$; maximum active concentration 0.3 $\mu\text{g/ml}$) are sufficient to liberate 5-HT from platelets. Calculation shows that 1 molecule of reserpine affects the release of hundreds of 5-HT molecules.

No release occurs when the incubation is carried out at 0° C.

SHORE *et al.* [1108, 1113] suggest that the release of 5-HT is not a simple displacement by reserpine and that a chemical ('denaturation' of the storage site) rather than a physical action is of major importance in the process.

Reserpine 10^{-5} M does not affect the 5-HT uptake by platelets suspended in saline alone, but inhibits the increased 5-HT uptake produced by addition of phosphate or potassium [1291b].

Spleen. 5-HT is released by reserpine from spleen as easily and completely as from blood platelets. This is in keeping with the probable localization of spleen 5-HT in platelets or platelet fragments [366, 379].

Gastro-intestinal tract. PLETSCHER *et al.* [932] first observed that reserpine produces a tremendous release of 5-HT from the small intestine of rabbits when given in intraperitoneal doses of 5 mg/kg. Following reserpine administration, the 5-HT content of the small intestine declines progressively for about 16 hours, finally reaching a value which is 15 to 20% of the normal. The concentration of 5-HT remains at this low level for about 16 hours and then increases slowly, reaching the normal value after about 5–10 days. As the dose of reserpine is reduced, decline in 5-HT becomes gradually smaller but it is still evident with as little as 0.25 mg/kg reserpine.

This observation has soon been confirmed and extended by other research workers. From the whole of the studies carried out in this field the following data clearly emerge:

a) 5-HT in the gastro-intestinal tract is released with greater difficulty than 5-HT in blood platelets and in the brain. With low single or repeated doses of 5-HT it is possible to obtain a nearly complete depletion of platelet 5-HT without affecting, even in the most sensitive animal species, the concentration of gastro-intestinal 5-HT [1264].

b) There are considerable species differences in the behaviour of gastro-intestinal 5-HT towards reserpine. Intestinal 5-HT is released easily by reserpine not only in rabbits, but also in guinea-pigs [86, 1269] and in hens [377], but much less so in rats and in lower vertebrates [919]. Even after high doses of reserpine 5-HT liberation does not exceed 25–50% in the rat [366, 1301, 630].

c) Striking differences may be observed in the action of reserpine on the 5-HT in the various sections of the gastro-intestinal tract. For example, in rabbits the 5-HT in the mucosa of the prepyloric part of the stomach is depleted by reserpine as easily as the 5-HT in the mucosa of the small intestine, whereas the 5-HT of the fundus is practically unaffected by the drug [1345, 939].

It is doubtful whether reserpine is capable of depleting the intestinal mucosa of 5-HT *in vitro*. In fact, the drug has failed to release 5-HT from the guinea-pig ileum suspended in oxygenated Tyrode solution even at a concentration of 100 $\mu\text{g/ml}$ [87].

Lung. 5-HT may be stored in the lung either in blood platelets or in mast cells. In the first case (rabbit) it is released by reserpine with ease, in the second (rat) with more difficulty [1268].

Other mammalian tissues. Extracerebral tissues of mammals other than the above contain 5-HT only in those animal species whose mast cells are capable of a biosynthesis of the amine, i.e. only in rats and mice. Mast cell 5-HT is released by reserpine with considerably greater difficulty than platelet, brain or intestinal 5-HT [907, 366].

Amphibian skin. Indolealkylamines of the amphibian skin (*Discoglossus pictus*, *Rana esculenta*, *Bufo bufo*) are not released by reserpine to any appreciable extent. The administered doses of the alkaloid ranged from 10 to 20 mg/kg daily for 3–6 days [738, 739, 919].

Posterior salivary glands of octopoda. No change in the 5-HT content has been observed following intracardiac injection of 5–10 mg/kg reserpine [919].

12.2 Effect of 5-HT releasers other than reserpine

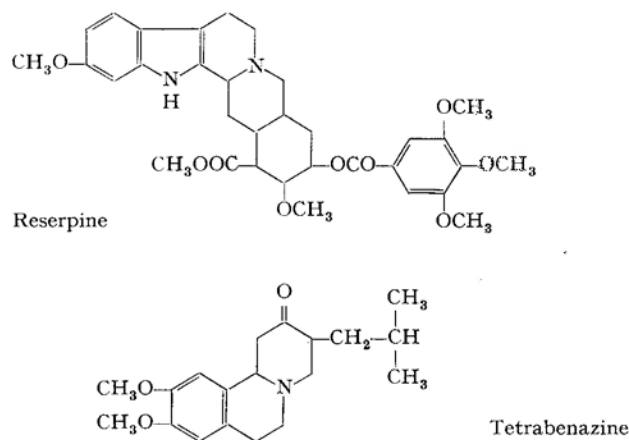
Besides reserpine, only rescinnamine, 11-desmethoxyreserpine and raunescine, among the *Rauwolfia* alkaloids, have caused release of 5-HT from the body depots [223].

The moderate depletion of gastro-intestinal and platelet 5-HT (35%) produced in rats by doses of raunescine above 5 mg/kg becomes more evident (50%) when the animals are pretreated with iproniazid [881, 630].

Of the semi-synthetic reserpine analogues, SU 3118 (carbethoxysyringoyl-methylreserpate) is a more powerful releaser of amines than SU 5171 (methyl-0-[3-dimethylaminobenzyl]-reserpate).

With low doses of these compounds (0.05 mg/kg for SU 3118), it becomes possible largely to deplete peripheral stores of catecholamines without producing appreciable changes in peripheral 5-HT and brain amines. With higher doses the concentration of brain amines is also reduced, but release of noradrenaline is again considerably more conspicuous than that of 5-HT [413, 386].

Tetrabenazine, the most potent representative of the 5-HT releasers of the benzochinolizine series produces, in doses of 20 mg/kg, i.p., only a slight reduction of 5-HT in the guinea-pig blood and no reduction of the 5-HT in the small intestine [1088].



However, urinary 5-HIAA excretion in rabbits could be increased by about 200% after administration of the drug [940].

BAS given to rabbits for three days at daily i.p. doses of 15 mg/kg apparently halves the 5-HT content of blood. A slight reduction of the amine concentration also occurs in the stomach and small intestine of mice, but no increase in the urinary 5-HIAA has been observed [1328].

LSD, BOL, phenobarbital, azacyclonol, Dibenamine®, and 48/80 do not release platelet 5-HT [223, 366]; chronic treatment with polymixin B reduces by 40% the skin 5-HT but only slightly the intestinal 5-HT [1196].

Chlorpromazine is ineffective on platelet 5-HT, while producing, according to WEST [1303] a moderate reduction in tissue 5-HT. However, it seems questionable whether this reduction is actual and not rather the expression of the anti-5-HT action of the chlorpromazine present in tissue extracts.

Compound 48/80, the well known histamine releaser, liberates only the 5-HT contained in mast cells, and 5-HT-liberation is generally a consequence of mast cell disruption. Depletion of 5-HT after repeated doses of 48/80 reaches 75% in the rat skin, but barely 10% in the rat spleen. If the animals are left to recover, there is a simultaneous regeneration of mast cells and recovery of the 5-HT and histamine content [901, 906].

It has been observed that subcutaneous morphine is absorbed about six times as quickly by 12-day-old rats as by fully-grown rats. This self-depression of subcutaneous absorption of morphine is interpreted as due to liberation of endogenous 5-HT, which is much more abundant in the skin of adult than in that of new-born animals [832].

12.3 Interference of reserpine with the biosynthesis of 5-HT

Reserpine, while producing a more or less conspicuous depletion of the 5-HT contained in the body stores, does not apparently interfere with the biosynthesis of the amine. This has been essentially proved by the unchanged excretion of 5-HIAA during chronic reserpine administration to human subjects and experimental animals [380, 1168].

Reserpine does not affect either the decarboxylation of 5-HTP [1037] or that of DOPA [684].

12.4 Biochemical consequences of 5-HT release and inhibition of binding of 5-HT by the tissues

a) *Increase of free 5-HT in blood plasma.* This may be observed both *in vitro* and *in vivo* provided that liberated 5-HT is adequately protected from rapid destruction by MAO.

Increase of free plasma 5-HT is achieved *in vitro* by incubating platelet suspensions with reserpine in an atmosphere of nitrogen. *In vivo*, plasma 5-HT is increased when the 5-HT liberator is administered after pretreatment with a MAO inhibitor.

The rise in plasma 5-HT concentration is accompanied by a similar decrease in platelet 5-HT.

In the absence of MAO block reserpine still liberates 5-HT from the platelets, but the released 5-HT does not appear in plasma [882].

With raunesine the increase of free 5-HT in plasma reaches a maximum after 1 hour (from 0.008 to 0.09 $\mu\text{g/ml}$) and disappears within 6 hours. 5-HT of plasma probably comes from platelets, but the possibility of release from other tissues cannot be excluded [634].

b) *Increase of urinary 5-HIAA excretion.* Following reserpine administration there is regularly a temporary increase in the urinary excretion of 5-HIAA, which coincides with the release of 5-HT from tissues and blood platelets. Thereafter, in spite of continuous administration of reserpine, the excretion of the 5-HT metabolite returns to premedication levels.

In man, doses of reserpine as low as 0.05–0.1 mg/kg produce, on the first day, an average increase of 25–50% in the excretion of 5-HIAA [1238, 1239, 438]. This increase reaches a maximum over the first 4 hours following reserpine administration and is high during the second 4 hours, but hardly significant thereafter [1198].

In dogs 0.1–3 mg/kg reserpine produces an increase in 5-HIAA excretion which reaches a maximum (400–500%) 2–4 hours after the drug has been given and persists for 8 hours or more [1107, 380].

In rats 5-HIAA excretion increases following 5 mg/kg reserpine by 30–40% during the first 3 hour-period, by 300–500% during the second period, by 40 to 300% during the third period, and returns to normal thereafter [366]. Finally, in rabbits 5-HIAA output reaches a maximum (800%) between the 2nd and

3rd hour subsequent to 2.5–3.5 mg/kg reserpine, and declines to normal after 5 hours [425].

In man, morphine and meperidine do not affect 5-HIAA excretion, nalorphine has a doubtful action [438], and large doses of benactyzine produce for 5 hours a striking drop in urinary 5-HIAA excretion without changing the total daily output of the metabolite [1261b].

The negative effect of chlorpromazine on 5-HIAA has been interpreted as being due to possible interference of metabolites of chlorpromazine with the colorimetric estimation of 5-HIAA [438].

c) *Reduction of the binding by tissues of the 5-HT originating from 5-HTP.* In rats pretreated with reserpine the yield of 5-HT from hind quarters, gastrointestinal tract and kidney perfused with 5-HTP is almost doubled, as compared with that observed in control animals [1037]. At the same time, reserpine sharply reduces the increase in tissue 5-HT levels usually produced by 5-HTP administration [172]. These facts are interpreted as being due to an inhibition of binding of formed 5-HT by the tissues.

12.5 5-HT as a mediator of the peripheral effects of reserpine

In order to avoid useless repetition we refer the reader for a critical discussion of this topic to the chapter dealing with the effects of reserpine and related 5-HT releasers on brain 5-HT.

Here it should be emphasized that still more than in the case of the central effects of reserpine, the importance of the participation of 5-HT in the production of the peripheral effects of reserpine is highly questionable. Catecholamine depletion seems to play a predominant role, and there is furthermore the possibility of depletion of other known and unknown amines.

This point of view, which is at variance with that previously defended by DEMING *et al.* [321], is now also being shared by BRODIE's group [413, 607c] on the basis of the observation that it is possible to deplete with carbethoxysyringoyl methyl reserpate (SU 3118) and methyl-o-(3-dimethylaminobenzyl)-reserpate (SU 5171) peripheral stores of noradrenaline without affecting peripheral stores of 5-HT or brain amines, and that this peripheral depletion is accompanied by bradycardia, a moderate fall in blood pressure and a marked diminution of the carotid occlusion reflex.

13. Indolealkylamines and the Central Nervous System

13.1 Pharmacological actions of 5-HT

13.11 Effects of 5-HT injected into the general circulation

a) *Effects on gross behaviour.* When given systematically 5-HT usually produces an effect resembling sedation and nervous depression. However, a mixed excitatory and depressory pattern has also been described, and in some cases a complete lack of effect has been observed.

In the rabbit, 5-HT in low doses (0.1–1 mg/kg) causes slight drowsiness, hypokinesia, less prompt reaction to stimuli and decreased attention reaction [64, 456, 840]; in very high doses, on the other hand, it causes excitation and restlessness [840]. Dogs seem to be rather insensitive to 5-HT, since intravenous doses varying from 0.06 to 2.5 mg/kg fail to provoke any change in behaviour [1024].

Rats and mice are depressed by 5-HT. In fact, 2–20 mg/kg of the amine, given subcutaneously produces in rats mild sedation and a general depression of motor activity [1113, 1106, 253] which lasts 3–4 hours and is accompanied by a rapid drop of body temperature and a 35–40% decline in oxygen consumption [588, 724]. The fall in body temperature is prevented by epinephrine (0.2 mg/kg) and is less pronounced in acclimatized animals [705]. It is, on the contrary, considerably enhanced (5–6°) by adrenalectomy [589]. In the presence of LSD in non-stimulating doses, the depression caused by 5-HT can be converted into stimulation [179].

In rats trained to self-stimulate their brain through electrodes in the posterior hypothalamus and midbrain tegumentum, 5-HT (4 mg/kg, s.c.), like other drugs, increases the threshold for rewarding brain stimulation or removes self-stimulation behaviour altogether [1172].

The cat responds to intravenous 5-HT with changes in behaviour very similar to those obtained by intraventricular injections: tendency to sit or lie down owing to muscular weakness, but no sleep or drowsiness [160]. In the monkey 5-HT intraperitoneally produces drowsiness, manifestations of anxiety with mood instability and, in high doses, hypotonia and paresis [65]. Whereas, like mammals, pigeons display a rapid hypothermia and depression after relatively moderate doses of 5-HT (5 mg/kg, i.v.) [587], frogs require enormous doses of the amine (at least 400 mg/kg into the dorsal lymphatic sac) to show areflexia, hypotony or respiratory changes [331].

According to CRONHEIM and GOURZIS [284b] infusion of small amounts (4 µg/kg/min) of 5-HT in reserpinized dogs produce a 'sedation' (reduced motor activity, lethargy, slow responsiveness to stimuli) greatly exceeding that produced by reserpine alone.

When injected into Siamese fighting fish subjected to a controlled stress situation, 5-HT increases the intensity but reduces the duration of the almost continuous activity of the fish [1213b]; similarly, in *Carassius auratus*, another fish, high intramuscular doses of 5-HT (50–200 mg/kg) produce a conspicuous inhibition of spontaneous motility with active or passive assumption of anomalous positions [1021]. Repeated administration of 5-HT leads to severe degenerative lesions in the motor nuclei of the cranial nerves and of the spinal cord [1020].

b) *Action on conditioned reflexes.* 5-HT (0.5–2.5 mg/kg i.v.) has been found by FLORU *et al.* [428] to cause in the dog a progressive inhibition of salivary conditioned reflexes and of reflex-conditioned behaviour as well as of experimental tics. 5-HT has also been found to change markedly the behavioral response of the animal to caffeine. These results are interpreted as the expression

of a massive decrease of the working capacity of cortical and subcortical centres. Chlorpromazine, LSD and BOL antagonize the effects of 5-HT; reserpine further enhances them.

The action of 5-HT on the conditioned escape reaction in rats is variable. Some investigators report a prolongation of the reaction time and an abolition of the reaction in 50% of the examined groups [513, 268], while others have been unable to see any inhibition of conditioned reactions even with 5-HT doses as large as 20 mg/kg [950].

c) *Action on convulsive seizures.* Generally, but not always, a protective action could be observed.

In mice, 20 mg/kg 5-HT given subcutaneously elevates the threshold to electroshock considerably, prolongs latency, and protects 50% of the animals completely against supramaximum electroshock [268]. However in the same animal species even enormous subcutaneous doses of 5-HT (up to 240 mg/kg!) have failed to affect camphor and leptazol seizures, or to interfere with the calmativ effect of reserpine on the convulsive attacks [116].

1 mg/kg 5-HT administered intravenously in the rabbit produces profound changes in the subsequent electroshock seizure. In fact, whereas the tonic phase remains unchanged, the clonic phase is greatly reduced or even lacking, being sometimes substituted by complete muscular relaxation [64]. A similar protective effect is shown by 5-HT (0.4–2 mg/kg i.v.) on strychnine and leptazol seizures. There is a transient inhibition of the EEG strychnine peaks and a clear attenuation of the convulsive attack. The anti-convulsant effect of 5-HT is blocked by previous administration of LSD, atropine and dihydroergotamine, and enhanced by reserpine and acetylcholine [410].

In the dog, 5-HT (0.1–1 mg/kg i.v.) does not affect the increase in cortical excitability produced by strychnine, but abolishes the predisposition to reflex epilepsy, and prevents or interrupts, to a varying extent, the epileptic attack, however produced [1058, 1059].

Similarly, the usual convulsant effect of strychnine in the frog is attenuated by 5-HT, although the amine increases the lethality of the animals [460].

d) *Action on spinal cord.* In unanaesthetized, vagotomized, high spinal cats intravenous injections of 5-HT (minimum active dose 5–10 µg/kg) characteristically produces a marked transient inhibition of the patellar reflex, followed by facilitation. Neuromuscular transmission in the quadriceps femoris muscle of the opposite leg is not affected. These effects are independent of arterial blood stabilization; they are markedly diminished by prior administration of chloralose. In intact cats changes in the patellar reflex induced by 5-HT are irregular and irreproducible, but they are usually characterized by some degree of depression [656, 657].

In slightly anaesthetized low spinal cats (T-13) intravenous 5-HT (50 µg/kg) causes a profound initial increase in amplitude and tone of the flexion reflex, which is followed by a gradual, marked depression. The knee jerk is only momentarily inhibited. LSD usually potentiates the stimulant phase and prevents the depressive phase of 5-HT [744].

Concerning spinal vasomotory mechanisms, BHARGAVA and BORISON [109] have observed that intravenous injections of 0.3 mg/kg 5-HT cause in the cat a depression of the threshold pressor response to medullary stimulation, without appreciably affecting the optimal medullary pressor response. When the dose is raised to 0.45 mg/kg the optimum medullary response is also depressed, but the drug still shows no effect on the pressor response to spinal compression. As shown by direct electric stimulation of the medulla oblongata, 5-HT has a depressant effect on supraspinal vasomotory mechanisms also.

e) *Action on electrical activity of the brain.* There is no concordance of opinion about the possibility of provoking in animals EEG changes after systemic administration of 5-HT.

ROTHBALLER [1005] has found that 5-HT (minimum active dose 2–12 μ g i.v.) produces in the cat first a brief EEG activation, then a longer, predominating period of deactivation, sometimes with abnormal slow waves, which is followed, after high doses of 5-HT, by a second brief period of activation. It has been suggested that the observed EEG changes occur via the activating reticular system.

However, still in the cat, doses as high as 1 mg/kg intravenously have failed to affect the excitability of the neurons in the amygdala to the hippocampus multineuronal pathway of the limbic system. This is viewed by REVZIN and COSTA [978, 980] as evidence against the hypothesis that 5-HT exerts its central effects through a direct synaptic action.

In the rabbit, MANTEGAZZINI [783] reports that 5-HT has no effect in subcutaneous doses of 2–10 mg/kg either on the EEG record of the resting animal or on the course of the arousal reaction. Only 15 mg/kg produces an appreciable activation of the EEG and a prolongation of the arousal reaction. No effect is noted on the EEG of the 'encéphale isolé' preparation of the cat.

Using, instead of the subcutaneous, the intravenous route, FERRO MILONE and GOMIRATO [410] obtained a desynchronizing effect on the EEG of the resting rabbit with 0.1–5 mg/kg doses of 5-HT.

More complex and partially at variance with preceding results are those of MONNIER *et al.* [456, 840, 855, 842] who have carried out the most extensive analysis on the effects of intravenous 5-HT on the electrical activity of the rabbit brain.

These Swiss investigators have found that small doses of 5-HT (0.1–1 mg/kg) produce a mixed arousal and relaxation pattern in which, however, the tranquillizing action on electrical activity is predominant. The specific action of 5-HT (exactly like that of reserpine) develops after a latency of 30–60 minutes and lasts over two hours. It is connected with an increase in excitability of the intralaminary thalamic system and a decrease in excitability of the reticular ascending system. Large doses of 5-HT produce an opposite, stimulating action, which persists in the isolated brain and is interpreted either as a massive action on all the neurones of the brain or as limited action on thalamocortical projections.

f) *Action on brain respiration and metabolism.* 2.5–25 mg/kg 5-HT given intravenously in the rabbit produce an important decrease in the brain con-

sumption of oxygen and glucose, a mobilization of brain glycogen and a reduction in brain production of pyruvate as well as an increase in the consumption of lactate and of inorganic phosphate [200, 203].

Lower doses of 5-HT (40–300 μ g) infused into the carotid artery of the cat do not affect either glucose uptake or oxygen consumption of the cerebral cortex [767].

The depressive action of 5-HT on brain oxygen consumption has been confirmed *in vivo* by BIANCHI [117] (25% reduction in the mouse brain, following 0.85 mg/kg 5-HT, s.c.), and *in vitro* by SALGARELLO [1029] and STARBUCK and HEIM [1169] (16–30% inhibition of oxygen uptake by 10^{-3} M 5-HT). However, chronic intraperitoneal administration of 5-HT produces, instead of a reduction, an increase in the oxygen metabolism of the rat brain [1030].

5-HT (1 mg/kg), LSD and BOL (0.25 mg/kg), all decrease the incorporation of 35 S-methionine into brain proteins. The ATP content of the rat brain is greatly reduced following administration of 5-HT alone, much less reduced in groups of animals receiving 5-HT plus LSD, and is increased in groups given 5-HT plus BOL [679b].

g) *Action on brain enzymes.* 5-HT (10 mM) shows no action either on the alkaline phosphatase or on the lactic or malic dehydrogenase of rat brain homogenates [259] nor does it inhibit *in vivo* oxidative phosphorylation in the rat brain, as determined by the uptake of P^{32} [742].

On the cholinesterase from rabbit caudate nucleus homogenates, 5-HT produces at final concentrations of 10^{-3} M, 5×10^{-3} M and 10^{-2} M an inhibition of 29, 63 and 100%, respectively.

Since marked inhibition occurs only at fairly high concentrations of 5-HT, APRISON [27] suggests that the action of the amine at normal physiological levels might be to compete with acetylcholine for acetylcholine-receptor proteins, whereas at elevated levels, such as after administration of large doses of 5-HTP, the amine would inhibit cholinesterase. Thus the sedative effect seen after moderate doses of 5-HTP might be the result of the block of normal acetylcholine action, whereas the stimulatory effect seen after large doses of 5-HTP might be due to the increased accumulation of acetylcholine after cholinesterase inhibition.

Results obtained on rat brain cholinesterase are substantially the same, provided that 5-HT is incubated with the brain homogenate before the addition of acetylcholine [1200].

h) *Action on the contractility and structure of oligodendrocytes and neurons.*

BENITEZ *et al.* [77, 1324] first observed that in tissue cultures 5-HT causes oligodendrocytes to contract strongly. 0.8 to 2 μ g/ml of the base proved to be ineffective, but 2 μ g/ml caused a contraction lasting 60–90 minutes. This effect has been confirmed by NAKAZAWA [860], who found that oligodendrocytes from mammalian thalamus cultivated *in vitro* display a marked increase in their contraction rates after 4 μ g 5-HT base/ml. The reaction to the amine is somewhat delayed and the contractions seem to be asynchronous; the removal of the drug is not accompanied by return to the basal rate of normal con-

traction. The stimulant effect of 5-HT is antagonized by the simultaneous administration of chlorpromazine.

GEIGER [466] has demonstrated that 5-HT acts not only on glia cells but also on the structure and motility of neurons. In fact 0.4–2 $\mu\text{g/ml}$ 5-HT base cause slow rhythmic pumping of the perikarya of neurones which forces cytoplasm down the axon. The nucleus enlarges at the beginning of contraction and aggregation of cytoplasmic granula is seen most strikingly in dendrites. Small undulating membranes appear on the fibres. The above morphological changes are inhibited by well-centrifuged Tyrode extracts of rabbit brain.

The interest of these observations is obvious, as they point to the possibility that one of the functions of 5-HT in the brain is to cause contraction of oligodendrocytes, which perhaps may be considered little stirring devices designed, among other things, to facilitate the circulation of extravascular fluid.

i) *Interference of 5-HT with the central effects caused by other drugs.*

α) Prolongation and potentiation of the sleeping time produced by hypnotics. This action of 5-HT was first described with accuracy by FORNAROLI and KOLLER [432, 434] and was soon confirmed by various other investigators [167, 1106, 1182, 1032, 391, 929, 179, 1340].

The potentiating effect of 5-HT has been studied particularly in rats and mice using hexobarbital as a hypnotic; but the hypnotic actions of cyclobarbitol, pentobarbital, thiopental, intranarcon, chloral hydrate, alcohol, mephensin and meprobamate are also prolonged by 5-HT. Negative or inconclusive results, on the contrary, have been reported for ethyl ether in rats [432, 929] and for ethylurethane and thiopental in rabbits [929].

To prolong sleeping time enormous doses of 5-HT (2–20 mg 5-HT base) are used generally. However, according to FORNAROLI and KOLLER [432] the minimum active dose of 5-HT is as little as 0.15–0.25 mg/kg, intravenously.

Similarly to 5-HT, both bufotenine and, to a lesser degree, tryptamine also are able to prolong chloral hydrate sleeping time considerably [390].

Bufotenine is more active than 5-HT in prolonging hexobarbital sleeping time in rats, and psilocybin still more active [489b].

The potentiating action of 5-HT on hexobarbital hypnosis in mice is blocked or markedly diminished by high doses (1–80 mg/kg) of LSD [1106, 179] and BOL [1032], but is further enhanced when LSD is given in the small dosage range ($\mu\text{g/kg}$ equivalent) that produces hallucinations in man. A similar enhancing effect is also produced by small doses of other hallucinogens (bufotenine, mescaline and ibogaine) and BOL [1035]. LSD does not suppress 5-HT potentiation of hypnosis produced by butabarbital and meprobamate [1340].

According to CAHN *et al.* [201, 202] the prolongation of barbiturate hypnosis produced in rats and rabbits by 5-HT is also antagonized by atropine, lysergic acid, adrenaline and Banthine®, and is favoured by chlorpromazine, Hydergin®, Neostigmine® and Regitine®. Similarly, β -tetrahydronathtylamine antagonizes the potentiating action of 5-HT, whereas its N-methy-N-propyl-derivative does not do so [913].

A few observations are necessary in regard to the interaction of 5-HT with hypnotics which, surprisingly enough, has raised such interest as seems to be surely out of proportion to its actual importance:

1) there is a long list of unrelated compounds which share with 5-HT the property of potentiating barbiturate hypnosis [391]; 2) there is important evidence suggesting that 5-HT prolongs the effect of hypnotics by virtue of its hypothermic action also. In fact, keeping mice at body temperature markedly reduces the prolongation effect of 5-HT on chloral hydrate sleeping time [391] and on pentobarbital sodium hypnosis [296b]; 3) finally, the enormous doses of 5-HT used to potentiate barbiturate hypnosis in rats and mice produce a complete renal block lasting for several hours. Thus, 5-HT could prolong the effect of certain hypnotics simply by delaying their renal excretion [391].

β) Interaction with LSD. 5-HT has been found to exert a certain prophylactic and therapeutic influence on LSD induced hypothermia in pigeons [587] and to antagonize the severe neuropsychic effects produced by LSD in monkeys [65].

However, only rarely and partially does 5-HT reverse the effects of LSD on spinal reflexes and the interaction of LSD and 5-HT has no significant effect on the knee jerk [744, 1278]. Similarly, the central actions produced by LSD in the rat do not change after 5-HT [251] and the chronic intoxication produced by LSD in the rabbit is not, or only poorly, ameliorated by high doses of 5-HT [930, 931].

Soon after the discovery of the powerful anti-5-HT action of LSD, the possibility was considered that the drug might display its tremendous hallucinogenic effects through inhibition of brain 5-HT.

This view should now be abandoned, as available evidence is decidedly against it, because, in fact:

1) in the series of LSD derivatives, anti-5-HT potency does not show any correlation with psychotomimetic potency. Derivatives more potent than LSD itself as 5-HT antagonists, such as BOL and UML, are practically devoid of any action on the psyche [243, 1006, 1007, 613];

2) no relationship has been found between 5-HT and mescaline and adrenochrome, which induce in man similar psychic changes to those induced by LSD [451];

3) ergometrine, which is a strong anti-5-HT compound, has no psychogenic properties at all. Yet it produces excitation and sham rage when given intraventricularly, and antagonizes the depressant effects of 5-HT, just like LSD [451];

4) BOL blocks, instead of reinforcing, the pyretogenic action of LSD, exactly as it blocks the pyretogenic action of 5-HTP. Thus, hyperpyrexia produced by LSD cannot be ascribed to 5-HT antagonism [597];

5) in a series of neurophysiological investigations no evidence of antagonism between LSD and 5-HT could be demonstrated on central synaptic activity. On the contrary, whenever, tested, LSD and 5-HT acted additively [965].

γ) Interaction with reserpine. Generally, 5-HT introduced into the general circulation reinforces the depressive and sedative actions of reserpine [65, 587]. However, emesis in pigeons, which can be induced by relatively small amounts of reserpine, is antagonized and not enhanced by the administration of repeated intramuscular doses of 5-HT [1070b].

δ) Interaction with other drugs. Whereas the catatonic action of *chlorpromazine* in the monkey is inhibited by 5-HT [65], the amine has little effect on the temperature response to chlorpromazine [587]. 5-HT (4.5 mg/kg i.p.) counteracts the increased motility produced by *Pervitin*® and *cocaine* in mice, and this action shows a linear relationship with the logarithm of the 5-HT dose [664]; *Ritalin*®, on the contrary, potentiates the tranquillizing effect of 5-HT in mice [1070b]. 5-HT (5–10 mg/kg s.c.) and tryptamine (100 mg/kg) prolong and enhance *morphine* analgesia in mice, but this potentiation does not appear to be specific [1119]. Finally, 5-HT (10 mg/kg s.c.) displays in mice a powerful protective action against convulsions provoked by pure *oxygen* administered at high absolute pressure [687].

13.12 Effects of 5-HT administered by close intraarterial injection

a) *Action on spinal cord.* CURTIS *et al.* [287, 345] observed that intraarterial injection of 20–85 μg 5-HT produces either a slight and transient depression of spinal reflexes from lumbar roots to corresponding ventral roots (stimulation frequency: 1–2 seconds), usually attributable to stimulation of peripheral receptors, or else a facilitation of these reflexes. Because excitation appears as early as 2 seconds after injection it is evidently not due to changes in blood pressure. There is no detectable effect on Renshaw cells.

SLATER *et al.* [1131] obtained somewhat divergent results. In vagotomized cats with the spinal cord divided at both C-2 and T-1, the intraarterial injection of 40–400 μg 5-HT base causes an initial increase and a secondary decrease in the flexion reflex. The anterior tibial muscle often remains contracted between stimuli, frequently obscuring the initial increase in reflex contraction. Shortly after 0.1 mg of LSD the stimulatory phase of the response is enhanced, whereas about one hour later or after a second dose of LSD the effect of 5-HT is blocked or considerably reduced.

Given into the carotid artery, in doses from 0.6 to 20 μg, 5-HT exerts a modulatory effect upon the amplitude of the stretch reflexes of the cat hind legs. With anaesthesia induced preponderantly with Dial® and urethane reflexes are inhibited, with anaesthesia induced preponderantly with chloralose facilitatory phases and ultimately pure facilitation is seen. Intravenous 5-HT is 10 to 15 times less effective than intracarotid 5-HT.

KOELLA and CZICMAN [668b] consider these results as evidence that 5-HT modulates the downward discharge from the reticular formation, as a consequence of an interaction with receptor sites in the brain stem.

b) *Action on the electrical activity of the brain.*

α) *Spontaneous electrical activity of the intact brain.* SLOCOMBE *et al.* [1134, 1135] found that the effects of both 5-HT (0.1–2 μg, intracarotically) and adren-

aline on the spontaneous electrical activity in the brain of rats is determined largely by the type of anaesthetic used. In penthotal-anaesthetized animals, profound reduction of the electrical activity in both frequency and amplitude is found in response to these drugs, while in ether-anaesthetized rats or in unanaesthetized spinal rats there is no depression of spontaneous activity, but in some cases an increase in slow wave amplitude superimposed on an unchanged fast activity. Both cortical and subcortical structures are equally affected, whereas respiratory and cardioregulatory centres are not significantly depressed. Inhibition of the transcallosally evoked potential is found to parallel diminishing spontaneous activity.

5-HT, LSD, adrenaline, noradrenaline and adrenochrome have no apparent qualitative differences in their effects, the 5-HT being from a quantitative point of view the most potent drug.

From the above data it has been concluded that the site of action of these compounds in the rat brain is in penthotal-sensitive nonspecific pathways (reticular formation of the brain stem and thalamus).

The spontaneous electrical activity of the cerebral cortex of the normal cat is unaffected by intracarotid doses of 5-HT base as high as 10–170 μg ; only higher doses (200–800 μg) produce a flattening of the EEG [283]. 5-HT is also completely ineffective in provoking the return of the normal arousal reaction blocked by the administration of 'ganglioplègiques centraux' [749].

β) *Spontaneous electrical activity of the 'encéphale isolé' and "cerveau isolé" preparations of the cat.* No effect could be elicited by intracarotid doses of 5-HT up to 2–45 μg on the desynchronized electrical activity either of the 'encéphale isolé' or of the 'cerveau isolé' [749, 784]; higher doses produce conspicuous reduction of the amplitude of the cortical waves [749].

However, when the EEG of the 'encéphale isolé' is synchronized following bilateral section of the trigemini, doses of 5-HT as low as 0.25–1 μg produce an evident arousal reaction. This remains unchanged following denervation of the carotid sinus or section of the brain stem at the distal end of the pons, but is abolished by section of the brain stem at the rostral end of the pons ('cerveau isolé') as well as by the administration of pentobarbitone, choralose or atropine. On the basis of these data, MANTEGAZZINI [784] suggests that 5-HT acts mainly on the pontine and perhaps bulbar section of the recruiting reticular formation of the brain stem.

γ) *Electrical activity of the isolated cerebral cortex.* DI STEFANO *et al.* [328] found that the potentials evoked by electrical stimulation from the neurologically isolated suprasylvian cortex of the cat brain are depressed by 2–4 μg 5-HT base given by intracarotid injection. Depression begins from 1 to 3 minutes following injection and persists for 4 to 8 minutes.

CREPAX and INFANTELLINA [283] observed that 5-HT potentiates to a slight degree the activity of cortical neurons in the isolated cortex preparation of the cat when injected into the carotid artery at low doses (8–40 μg) or applied topically in low concentrations (10^{-4} – 10^{-3}) while producing clear depressive effects at higher doses (up to 1 mg base) or concentrations (up to 10^{-2}).

Finally, RECH and DOMINO [973] noted that intracarotid or intravenous (!) injections of 5-HT (8–25 μg) decrease the frequency of the strychnine or α -tubocurarine spiking in the dog isolated cortical area. However, the same effect could be elicited also by adrenaline and noradrenaline and in any event the action of these agents on spiking frequency was quite variable and often not significant.

δ) *Evoked electrical transcallosal response.* MARAZZI and HART [786, 787] first studied cerebral synaptic transmission by recording the postsynaptic electric response evoked by presynaptic electrical stimulation in the relatively simple transcallosal pathway connecting symmetrical points in the right and left optic cortex of the cat.

They found that intracarotid doses of as little as 1 μg 5-HT produce an evident synaptic inhibition. The effect is achieved within 30–40 seconds from the injection, reaches a maximum in about 1 minute and recedes after 3 minutes. Neither carotid body nor carotid sinus interfere in its production [992b]. Several other drugs (adrenaline, mescaline, adrenochrome, LSD) display the same inhibitory action as 5-HT but, with the important exception of bufotenine (which is twice as potent as 5-HT), they are less potent than 5-HT: LSD and adrenaline, for example, are 6 to 8 and 25 to 30 times less potent than 5-HT, respectively.

The synaptic inhibition is prevented by prophylactic doses of the tranquilizers chlorpromazine, reserpine and azacyclonol in amounts which in themselves do not alter synaptic transmission [787, 698] but is potentiated by iproniazid (5 mg/kg, intracarotid). Iproniazid alone (10 mg/kg) reproduces the picture of 5-HT action, namely, a reduction of both the evoked response in the optic cortex of the cat and the electrocorticogram recorded from the same electrodes [499, 498, 787b].

KOELLA *et al.* [668, 667, 1146] have thoroughly re-examined the problem of the inhibitory effect of intracarotid 5-HT on optically evoked cortical potentials. Having found that the effects of 5-HT (1.25–10 μg , or repeated doses of 5 μg) vary depending upon whether the drug is injected into a carotid artery with a normally innervated or denervated carotid sinus and whether the injection is given into the ipsi- or the contralateral artery, with respect to recording, these investigators conclude that 5-HT exerts its inhibitory effects upon the cortical potentials via at least three receptor systems: 1) by stimulating receptors in the carotid sinus area and thus initiating modulatory impulses in the diffuse unspecific ascending system, 2) by stimulating receptors in the brain stem and initiating ascending unspecific modulatory discharges and 3) by a direct chemical effect upon the specific sensory pathways.

There are several points in the KOELLA hypothesis which are not clear and persuasive. First of all the statistical and algebraic analysis of their data is rather forced, and secondly it is difficult for the reader to understand whether the investigators are or are not of the opinion that 5-HT is capable of passing the blood-brain barrier.

ε) *Electrical responses evoked in cortical and subcortical structures by somatic sensory nerve stimulation.* MALCOLM [771] observed that following intracarotid

injection in the cat of 80–130 μ g 5-HT base there is an initial depression of the responses evoked in the primary sensory area by somatic sensory nerve stimulation, followed, after 12–16 minutes, by an enhancement. Enhancement never completely disappears, and with subsequent injections the response to 5-HT becomes relatively less. The action of 5-HT on evoked responses in all the deeper structures (thalamus) reached by the somatic afferent volley is solely one of long-lasting depression. MALCOLM suggests that the phase of diminished response would appear to be associated with the vascular effects of intracarotid 5-HT, more precisely with oedema formation, while the relatively long-lasting phase of enhancement would represent the net, specific action of 5-HT on the primary sensory area, provided, of course, that enhancement is not interpreted as a release phenomenon.

In intracarotid doses of up to 1.2–1.7 mg, 5-HT fails to affect the geniculate response to stimulation of the optic nerve. Similarly, 5-HT administered before or after LSD causes no consistent change in the LSD effect [387d].

13.13 Passage of 5-HT through the blood-brain barrier

UDENFRIEND *et al.* [1228] have reported that no detectable increase in the brain 5-HT levels could be produced in dogs and rabbits after peripheral administration of very large amounts of 5-HT (27 mg/kg 5-HT base). SOUTHERN [1149] did not succeed in provoking any rise in the level of 5-HT in the cerebrospinal fluid of a human subject given large amounts of 5-HT by intravenous infusion. We are able to supply substantial confirmation of these negative results. In our experiments, 10–20 mg/kg 5-HT given intravenously in pregnant rabbits failed, in sharp contrast to 5-HTP and 4-HTP, to modify either the 5-HT content of the mother's brain or the 5-HT content of the brain of the foetuses [386c].

At variance with the above observations, SHORE *et al.* [1113] found that 15 minutes after the intraperitoneal injection of 45 mg/kg 5-HT, the brain level of the amine in mice rises from 0.9 to 1.2 μ g/g.

Similarly, COSTA and APRISON [273, 276] observed, in cross circulation experiments on rabbits, that 2–4 mg of 5-HT injected into the internal carotid artery provokes a significant increase of 5-HT in some specific areas of the brain (telencephalon, midbrain and cerebellum) of the recipient animal. They conclude that the impermeability of the blood brain barrier does not appear to be absolute.

However, the most surprising results seem to be those of KAERKI and PAA-SONEN [631], who have reported that 10 mg/kg of 5-HT injected into the tail vein produces in rats a conspicuous increase in the 5-HT level of the brain (from 0.5 to 1.2 μ g/g) lasting 40 minutes. This, of course, would signify an extremely easy permeability of the blood brain barrier for 5-HT, perhaps greater than that for 5-HTP.

Actually, the negative results obtained in dogs and rabbits seem to be more persuasive than the positive results, and the bulk of experimental evidence available is in favour of the view that 5-HT does not pass in detectable amounts

through a normal blood-brain barrier. The experiments of COSTA and APRISON do not contribute to the solution of the problem since the enormous, mg doses of 5-HT injected directly into the carotid artery surely produce profound changes in caliber and permeability of cerebral blood vessels, thus probably altering the permeability of the blood-brain barrier. The same objection is valid for the experiments of KAERKI and PAASONEN. In addition, it should be kept in mind that in rats and mice the brain vessels seem to be particularly sensitive to 5-HT [181] and that 5-HT may be absorbed, within the central nervous system of these species, by cells other than nervous cells. We refer to mast cells and possibly to other connective cells.

Perhaps a definite solution to the problem here under discussion will be provided by experiments with radioactive 5-HT, similar to those carried out by WEIL-MALHERBE *et al.* [1283] with tritium-labelled adrenaline. It is true that McISAAC and PAGE [810] have found a significant radioactivity in brain tissue of rats and rabbits given intraperitoneally ^{14}C -5-HT (17 and 2 mg base/kg, respectively), but the investigators could not exclude the possibility that this radioactivity might be due to the entry of metabolites rather than to 5-HT. It should be added that, precisely in rats, McISAAC and PAGE found in brain tissue a very variable radioactivity, barely corresponding to 0.2–4% of that found in blood, i.e. a radioactivity which could easily be accounted for by the blood content of the brain tissue.

In sharp contrast to 5-HT, it appears that systematically injected tryptamine is capable of penetrating with ease and rapidity into the rat brain. In fact, 60 to 75 seconds after the intravenous injection of 5 mg/kg tryptamine in animals pretreated with either tranlycypromine or iproniazid, the brain concentration of the amine is increased from 0.03 to 0.173 $\mu\text{g/g}$ [519].

When given systematically 5-HT displays general behavioural effects, potentiates the barbiturate hypnosis and provokes changes in conditioned reflexes, in spinal reflex excitability, in brain electrical activity, and so on.

Does this mean that these effects are brought about by a direct action of the drug on neurons or synapses within the central nervous system? Is it reasonable to draw from these effects any conclusion about the significance of brain 5-HT? Definitely not!

It is rather astonishing to see that in many experiments 5-HT has been administered at enormous dose levels, without paying the least attention to what happens to blood pressure and body temperature and without considering the many other peripheral systemic effects of the substance. When blood pressure has fallen nearly to shock levels, when body temperature has dropped by several degrees, and the animal is disturbed by gastro-intestinal distress, it certainly is imprudent to speak of mild sedation or of general depression of motor activity.

But even in the case of 5-HT being used at more reasonable dosage levels it should always be kept in mind

α) that so far persuasive evidence demonstrating that 5-HT is capable of passing a normal blood-brain barrier is lacking.

β) that 5-HT has been shown to exert a conspicuous vasoconstrictor action on cerebral vessels [64, 771] and to provoke changes in their permeability, thus possibly affecting brain neurons through anoxia. Microgram doses of 5-HT, such as are generally injected into the carotid artery, are apparently low, but in this respect it should not be forgotten that intraarterial infusion of doses of 5-HT base as low as 0.4 µg/min, for 5 to 10 minutes, causes a 30% fall in blood flow in the human forearm [496] and that doses of 5-HT base as low as 0.04–4 µg injected into the dog coronary arteries are capable of increasing coronary flow without affecting the systemic blood pressure [1085]. Moreover, it should be emphasized, especially when dealing with intraarterial injections, that we know practically nothing about the regional circulation of the brain, and that it is quite possible that a drug may produce vascular effects in restricted areas of the brain with no appreciable change in the total venous effluent of the brain and, of course, with no change in the systemic blood pressure.

γ) that 5-HT may act on the CSN indirectly through nervous discharges evoked from the carotid sinus receptors [668], from peripheral receptors in the intestinal wall [185], in the lung [891, 336b] or in the heart [888b], as well as from known and unknown vascular receptor or trigger zones situated close or within the central nervous system, but outside the blood brain barrier which encloses other parts of the brain. The existence of such areas has recently been confirmed by BRODIE *et al.* [176] who found that both lobes of the pituitary, the area postrema and the intercolumnar tubercle differ from other parts of the brain in their power of absorbing organic compounds from the blood.

δ) finally, that no central effects of 5-HT have been observed in patients suffering from carcinoidosis, a morbid syndrome characterized by continuous or paroxysmal hyperproduction of 5-HT.

Catecholamines bear many resemblances to 5-HT. Because this is so, it seems important to remember that tritium-labelled adrenaline has been shown to be incapable of crossing the blood brain barrier in the cat, except perhaps to a small extent in the hypothalamus (concentrations of labelled adrenaline have not exceeded 0.025 µg/g! [1283]), and that MANPEGAZZINI *et al.* [785] could not duplicate with intracarotid or intravertebral injections of adrenaline or nor-adrenaline the EEG arousal reaction produced by intravenous catecholamines. This suggests that perhaps the EEG effects of catecholamines are not direct, but rather may depend on the vascular actions of the drugs.

It is true that in the experiments of DRASKOCI *et al.* [339] adrenaline infused intravenously in the cat at a rate of 40 µg/kg/min was found to be capable of penetrating into the cerebral ventricles and even more so into the subarachnoidal space; but in this connection it should be observed that the doses of adrenaline administered were very high, that in cerebrospinal fluid of cavities not perfused with saline, adrenaline was found only after prolonged infusion (hence the possibility of a change in permeability of the blood vessels, following vasoconstriction and anoxia), and, finally, that it is quite possible that the adrenaline in the subarachnoidal space originated from the meningeal vessels and passed from these directly into the subarachnoidal space.

Of course, the above objections cannot rule out completely the possibility that a smaller number of the central effects produced by 5-HT administered systematically are due to a direct effect of the drug on neurons or synapses within the central nervous system.

13.14 Effects of 5-HT injected into the lateral ventricles

a) *Behaviour effects.* FELDBERG and SHERWOOD [397b] were the first to introduce 5-HT into the lateral ventricles of the cat brain through a permanent cannula. A dose of 10 μg of the drug has no detectable effect; 100–200 μg after a latency of about 6 to 8 minutes produces a pronounced tendency to sit or lie down. The cat is not sleepy or drowsy, the eyelids remain wide open and blinking movements are rare. Besides muscular weakness, tachypnoea and bursts of profuse salivation are observed.

These observations have been fully confirmed by other investigators [450, 160 1260, 453, 1087, 1186].

The lethargic state produced in the cat by intraventricular injections of 5-HT is antagonized by parenteral administration of LSD (0.8 mg), ergometrine (1.5 mg), morphine (1–3 mg), methadone (10 mg), β -tetrahydronaphthylamine, amphetamine (15 mg) and methylamphetamine. Conversely, BOL, methyl-medmain (6 mg) and 5-benzyloxygramine (2 mg) are ineffective. Surprisingly enough, amphetamine seems to intensify the depressant action of 5-HT when injected intraventricularly (0.5 mg) instead of subcutaneously [450, 1260, 453]. Intraventricular LSD (15 μg), mescaline (0.3–1 mg) adrenochrome (0.2–0.5 mg) or adrenolutine (0.3–0.65 mg) do not change the behavioural reaction to 5-HT [1087]. It has been noted by VOGT [1260] that all drugs found to overcome the sedation produced by 5-HT also cause stimulation of the sympathetic centres.

According to WOOLEY [1320] the changes in behaviour produced in mice by intraperitoneal LSD can be prevented in 25% of the animals by the intraventricular injection of 5-HT (8 μg) plus carbamylcholine (0.2 μg). 5-HT alone is ineffective and similarly ineffective is the combination of the two drugs when given intraperitoneally.

b) *Effects on the electrical activity of the brain.* SCHWARTZ *et al.* could find no significant changes in the EEG following intraventricular injection of 75–500 μg 5-HT in conscious trained cats. Other investigators [159, 160, 1260], on the other hand, have observed that 200–250 μg 5-HT produces an increase in slow rhythms of the cerebral cortex, which still respond to sensory stimuli with a normal alerting of electrical activity. The effect, which is not very pronounced, takes about 30 minutes to become fully established and only after several hours does recovery take place.

No changes in the electrical activity of 'encéphale isolé' preparations could be noted following intraventricular injections of 5-HT [160].

Because intraventricular adrenaline (100–200 μg) produces exactly the same EEG effects as 5-HT, HANCE and BRADLEY [543] believe that these effects are not specific. Moreover, on the basis of results obtained with a combination of

5-HT and LSD, the same investigators suggest that there exists a possible synergism between the central effects of the two drugs.

This opinion is shared by SAWYER [1055b] who has observed that intraventricular injections of 0.75 mg 5-HT or 1 mg LSD in the flaxedil-immobilized cat exerts profound and reproducible effects on the EEG of subcortical structures. In certain regions the effects of the two drugs are parallel and additive.

According to BRADLEY, intraventricular 5-HT scarcely affects the activity of the cerebral cortex, but is more likely to interfere with the descending influences of the reticular formation of the brain stem; according to SAWYER the neural correlates of the effects of 5-HT may be found in the rhinencephalic-basal ganglia areas and their projections.

c) *Effects on conditioned responses.* Intraventricular injection of 0.27 mg 5-HT plus iproniazid, whether given before, with or after reserpine fails to modify the reserpine effects on the conditioned avoidance responses of the cat, or on peripheral autonomic signs [621].

d) *Effects on vascular reflexes and on vasomotor activity.* BHARGAVA and TANGRI [110] have found that injection of 0.5–2 mg 5-HT into the dog cerebral ventricles causes hypotension, inhibition of the pressor response to occlusion of the carotid artery and inhibition of the pressor or depressor response evoked by electrical stimulation of the central end of the cut vagus nerve.

These results have been confirmed by McCUBBIN *et al.* [812] who could demonstrate that reserpine (0.5–2.5 mg), 5-HT (0.4–4 mg base), noradrenaline, 5-HTP (0.25–5 mg) and DOPA (0.5–10 mg) have qualitatively the same cardiovascular effects when injected into a lateral ventricle or into the cisterna magna of the dog. On a weight basis noradrenaline is the most active amine, and 5-HTP is more active than 5-HT or DOPA. Central injection of Catron® markedly augments the cardiovascular effects of the amines and of reserpine as well.

Because cooling of cerebrospinal fluid also inhibits carotid occlusion response and causes hypotension and bradycardia, whereas vasodilator drugs and warming of the cerebrospinal fluid elicit opposite effects, it has been tentatively concluded that the action of 5-HT and noradrenaline on central vasomotor activity is due to changes in local blood flow [629b].

At partial variance with the above findings, GINZEL [485] observed that in the cat repeated intraventricular injections of 10–200 μ g 5-HT do not of themselves exert any influence on vascular reflexes arising from the carotid sinus area, nor do they affect the inhibitory action of LSD.

13.15 Effects of 5-HT injected into the cisterna magna

5-HT introduced into the cisterna magna of the conscious dog in doses of 40–900 μ g/kg, provokes in the majority of cases a cataleptic picture, appearing after a latency of 10 to 60 minutes, and lasting up to 6 hours. Vegetative symptoms are not intense and consist of salivation and repeated micturition.

Animals subjected to multiple injections of 5-HT (maximum total dose: 23 mg) do not display, after treatment is discontinued, any change either in behaviour or in vegetative functions [1024, 143].

Intravenous (20–60 $\mu\text{g/kg}$) or intracisternal (10–15 $\mu\text{g/kg}$) LSD exerts only a partial antagonistic action towards 5-HT; intravenous methylamphetamine (0.5–3 mg/kg), on the contrary, immediately interrupts 5-HT catalepsy [1023, 459].

For the appearance of catalepsy it is necessary for 5-HT to penetrate from the cisterna into the 3rd ventricle, thus contacting the paraventricular centres; otherwise the drug is ineffective [1022].

A cataleptic picture can be provoked also in frogs, when 5-HT (25–750 $\mu\text{g/g!}$) is injected into the lymphatic sac directly communicating with the brain [331, 332].

In rabbits the intracisternal injection of 0.5 mg 5-HT causes hyperglycaemia and depletion of the adrenal medulla. These effects are potentiated by iproniazid [594].

The most serious objection to the actual importance and to the interpretation of the experiments just described is that raised by GADDUM and VOGT [450]: 'It must be remembered that there is no evidence that the effects of intraventricular 5-HT are exerted directly on neurones and not mediated through a constriction of cerebral blood vessels in regions near the ventricles. The depression produced by injecting large doses of adrenaline into the ventricles bears much resemblance to the effect of intraventricular 5-HT and vascular effects may play some part at least in the response to both drugs when they are administered by this route.'

This view, also shared by BRODIE [170] and others [771], still maintains its full validity. In fact, one can hardly avoid thinking that the relatively enormous doses of 5-HT introduced into the ventricles are capable of provoking profound changes in calibre and permeability of blood vessels in the ventricular wall. In cats, for example, MALCOLM has observed a long-lasting and not easily reversible oedema of the cortex following intracarotid injection of 200–300 μg 5-HT, a dose equal to or less than that injected into the ventricles.

13.16 Effects of 5-HT injected into the brain tissue

HALEY [537, 538] observed that 5-HT injected intracerebrally in mice, in doses of 4–13 μg (0.05 ml liquid), causes an autonomous nervous system discharge (tachypnoea, micturition, defaecation, piloerection) together with central depression and scratching. Normal activity is resumed in 15 to 240 minutes, depending on the dose. Intracerebral LSD, acetyl-LSD and 1-methyl-LSD (10 μg) are able to block the central effects of 5-HT.

OLDS and OLDS [870, 871] found that on injection or self-injection of 5-HT into the rat hypothalamus (0.5–1.5 μg in 0.5 μl liquid), the animals quickly lose muscular tone and apparently go to sleep. An identical effect is produced by acetylcholine. The animals can be brought back to their feet by adrenaline, whereas noradrenaline and ATP have no observable effect.

Results obtained with injections of a drug into the brain substance arouse extreme perplexity. In fact, it is very difficult to exclude the possibility that

5-HT acts on neurons not directly and specifically, but only indirectly through effects displayed on blood capillaries or on neuroglia or, more roughly, through the lesion induced by the microinjection [972].

This criticism is considerably less relevant in the case of the recent, skillful experiments of CURTIS and KOIZUMI [287b]. 5-HT was applied electrophoretically, in high concentrations, into the cellular fluid surrounding neurones in the brain stem (upper mesencephalic reticular formation and inferior colliculus) of the unanaesthetized decerebrated cat. Spike responses of neurones were recorded by means of double or five barrel electrodes. The result was that 5-HT (like noradrenaline and d-tubocurarine) failed to affect the response of the cells under investigation, thus making it unlikely that the amine has a transmitter function in the mesencephalic reticular formation.

13.17 Effects of 5-HT applied topically to the cerebral cortex

5-HT applied topically to the exposed cerebral cortex of cats and rabbits, in concentrations of 0.5 to 1 %, greatly diminishes or eliminates, for at least 30 minutes, the negative wave direct cortical responses. The effect is produced both when 5-HT is placed on the recording site and when it is placed on the stimulated site. According to OCHS *et al.* [868c] the above results indicate a generalized membrane effect of 5-HT on apical dendrites of pyramidal cells.

13.18 Effects of 5-HT on the central nervous system of molluscs

Two types of nervous cells with different and specific pharmacological properties are present in the central nervous system of *Aplysia depilans*: cells H having inhibitory input, and cells D having only excitatory input. 5-HT, in concentrations of 10 µg/ml nutrient liquid, excites both types of cells, but its effect is more than 10 times more intense on D-cells [470b].

13.2 Pharmacological actions of indolealkylamines other than 5-HT

13.21 Bufotenine and related 5-hydroxyindolealkylamines

a) *Action on performance of trained rats.* Both bufotenine (8–27 mg/kg, i.p.) and LSD (0.3–0.5 mg/kg i.p.) produce in rats confusion concerning the escape from noxious stimuli, pronounced peripheral vasodilation, change in normal posture (dragging the abdomen on the cage floor) and prolongation of climbing time. However, bufotenine is 25 times less effective than LSD and has a longer duration of action. Tolerance to repeated injections has been observed. The effect on climbing time produced by submaximum doses of bufotenine is altered by pretreatment of animals with reserpine, iproniazid or 5-HTP. Pretreatment with 5-HTP, for example, inhibits highly effective doses of bufotenine and LSD.

In contrast to LSD, bufotenine does not cause salivation, nor does it increase the frequency of urination and defaecation [768, 769].

b) *Behavioural effects in dogs.* 1–4 mg/kg bufotenine, given intravenously or intraperitoneally, produces salivation, muscular weakness with pseudo-

paraplegic splaying out of the hind legs, dissociated behaviour with indifference to noxious stimuli and to attack by other dogs, tongue lapping, hyperesthesia and howling [389b]. The symptoms, lasting in some cases for hours, are more pronounced when the same bufotenine dose is given into the vertebral artery, than when it is injected into the carotid artery. These differences depend on the different areas of the brain irrigated by the vertebral and carotid blood, respectively [580].

It has been suggested by HIMWICH and COSTA [580c] that the bulbar portion of the reticular formation or other structures in the medulla might be the site of action of bufotenine injected into the vertebral artery.

c) *Behavioural and neurological effects in the monkey.* Bufotenine (5 mg/kg i.v.) produces in the monkey a syndrome characterized by gross sensory disorder in the absence of a clear defect in muscular power, and a marked degree of tameness. While vestibular eye movements and reactions to auditory stimuli are grossly normal and pupillary light reflex is present, deep tendon reflexes are hyperactive, reaction to painful and visual stimuli is absent and locomotion is ataxic [387c].

d) *Action on monosynaptic spinal reflexes.* Like 5-HT, but less intensely, bufotenine and bufotenine O-phosphate inhibit the monosynaptic patellar reflex in the cat. The minimum active dose is 50 µg/kg, intravenously.

The corresponding 4-hydroxyindolealkylamines (psilocin and psilocybin) have an opposite effect [1280].

e) *Action on spontaneous electrical activity of the brain.* In the rabbit, bufotenine (0.3 mg/kg, i.v.) causes the disappearance of slow wave high voltage activity and introduces an enduring 'alert' desynchronized pattern. Contemporaneously there is an intense stimulation of smooth musculature [981].

In the cat, intracarotid injections of 0.2 mg/kg bufotenine decreases the amplitude of spontaneous cortical activity [387b].

f) *Action on evoked electrical activity in the cat's visual system.* Bufotenine injected into the cat carotid artery has no effect, in doses up to 5 mg/kg, on the amplitude of the optic tract response to photic stimulation of the retina. Similarly, this amine does not decrease the cortical response to optic radiation stimulation.

In contrast to the high resistance to bufotenine of the specific cortical response, the thalamic relay station is quite sensitive to the substance. In fact, doses of 0.2 mg/kg bufotenine cause a marked depression of the postsynaptic response to shocks applied to the optic nerve, recorded in the lateral geniculate nucleus. The latency of the maximum effects varies from 20 to 60 seconds and the maximum decrease of postsynaptic spike amplitude is 80%. The amplitude begins to increase within 10 minutes and reaches the preinjection amplitude within one hour.

In cats decerebrated by intercollicular section the dosage required to produce an equivalent decrease is 2-3 times greater. The electrophysiological effects of bufotenine are independent of any blood pressure or respiratory changes.

5,6-dimethoxybufotenine (3–4 mg/kg, intracarotid) is inactive on synaptic transmission in the lateral geniculate nucleus [387b, 387d].

g) *Action on brain enzymes.* Bufotenine has proved to be a very active inhibitor of brain cholinesterase [47] and a light inhibitor of lactic dehydrogenase [259]. Brain alkaline phosphatase is activated by the drug [47]; oxidative phosphorylation in preparations of brain mitochondria is, on the contrary, unaffected by concentrations of bufotenine as great as 10^{-3} M [47].

13.22 5-Methoxyindolealkylamines

O-Methylation of hydroxyindolealkylamines increases their ability to cause behavioural mistakes in rats conditioned to escape from an electric shock in response to a warning auditory stimulus. At dose levels of 0.1 mM/kg 5-methoxy-N,N-dimethyltryptamine is three times more effective than bufotenine and 5-methoxytryptamine is at least as potent as 5-HT. Melatonin does not cause any behavioural disturbance in doses up to 0.2 mM/kg [474c].

13.23 Psilocybin and other 4-hydroxyindolealkylamines

a) *Behavioural effects in mice.* Psilocybin provokes in the dancing mouse changes in behaviour similar to those produced by LSD. The intraperitoneal doses required to cause arrest of the circular motion are 5–10 mg and 0.1 mg/kg, respectively.

Psilocybin acts more promptly than LSD, and induces a more intense reactivity to noxious stimuli and a greater vigilance. Neurovegetative symptoms (horripilation, tremor, salivation) are less pronounced [315].

b) *Behavioural and EEG effects in the rabbit.* Psilocybin (1.5–5 mg/kg i.v.) develops in the waking rabbit a marked activation of the somatic behaviour and of the electrical brain activity. MONNIER [841] suggests that this stimulating action should be ascribed to a depression of the mediotthalamic recruiting and moderating system rather than to an activation of the reticular arousal system.

Predominant neurovegetative symptoms, showing sympathetic stimulation, are mydriasis, elevation of blood pressure, piloerection, short-lasting bradycardia, prolonged hyperpnoea and hyperpyrexia [1279].

c) *Action on spinal monosynaptic reflexes.* In contrast to the corresponding 5-hydroxyindolealkylamines, both psilocybin and psilocin facilitate in the cat the patellar reflex in intravenous doses as low as 5–10 μ g/kg [1280]. 4-HT, however, like 5-HT, temporarily blocks the patellar reflex [1281].

Action on brain enzymes. The inhibitory effect of psilocybin and 5-HT on gray matter cholinesterase is identical. This indicates that there is no correlation between the *in vitro* anticholinesterase activity of indolealkylamines and their hallucinogenic effect [1354b].

d) *Effects on web-building behaviour of spiders.* Six g/kg synthetic psilocybin given to spiders per os in sugar water 12 hours before web-building time stopped web-building completely for 24 to 40 hours. After 600 and 300 mg/kg of psilo-

cybin, 5 and 11 % of the animals, respectively, built the next day. 150 mg/kg psilocybin given to *Araneus diadematus* on 4 different days caused a delay in web-building and webs showed important changes in their aspect. Spiders behaved under the influence of psilocybin as if they had become heavier [1316b].

13.24 Tryptamines

a) *Convulsant action in rats.* Intravenous tryptamine evokes convulsions in rats. The minimum active dose is approximately 5 mg/kg, the ED 100 is 40 mg/kg. The convulsant action of tryptamine is potentiated by MAO-inhibitors and antagonized by LSD, BOL, chlorpromazine, prochlorperazine and trifluoperazine. 5-HT and 5-HTP fail to antagonize tryptamine convulsions, exactly as they fail to antagonize electroshock seizures [1192, 1194].

According to GREEN and SAWYER [519], occurrence, duration and relative intensity of clonic convulsions produced by injections of tryptamine into rats pretreated with MAO inhibitors are correlated with increased concentration of the amine in the brain.

b) *Reversal of reserpine sedation.* An infusion of 1.5 mg/kg/min tryptamine into reserpinized rabbits effectively antagonizes the ptosis, myosis and depression induced by reserpine. This action is markedly potentiated and prolonged by combination with iproniazid [1192, 1194].

c) *Behavioural effects in the dog.* After administration of 2 mg/kg N,N-diethyltryptamine the animals display activation of their orientation, together with intense vegetative manifestations such as mydriasis and changes in respiration. The dogs look like a patient suffering from hallucinations. These effects last two hours and their intensity fluctuates periodically, just as in human subjects. Concerning conditioned reflexes which are built up in dogs beforehand, there is seen a lessening in discrimination capacity and a lengthening of the time that elapses between the acoustic stimulus and the conditioned response [1025].

d) *Action on synaptic transmission in the lateral geniculate nucleus of the cat.* Both tryptamine and dimethyltryptamine depress geniculate response to optic nerve shock. However, although the percentage decrease of the post-synaptic response at the peak effect of tryptamine is of the same order as that caused by its N,N-dimethyl derivate and by bufotenine, the duration of the effect in the case of tryptamine is considerably less (3–6 minutes) than with the other two agents (30–60minutes).

The tryptamines do not reduce the cortical response to stimulation of geniculate radiation [387d].

e) *Action on the cerebral electrical activity of the rabbit.* Intravenous injections of tryptamine (15 mg/kg) cause a flattening of the electrocorticogram and the development of a 30–40 c/sec discharge in the rhinencephalon, similar to that provoked by 5-HT. Concomitantly, muscular hypertonia, diffuse tremors and arterial hypertension may be seen. Infusion of tryptamine, at a rate of 1 mg/min for a period of 90 minutes fails to evoke this pattern [332b].

f) *Effects of α -methyltryptamine.* With intraperitoneal dosages of 2–5 mg/kg, the drug produces in rats an increase of brain 5-HT, and at higher dosage levels (20 mg/kg) also a reduction of brain 5-HTP decarboxylase activity. Simultaneously, rats and other animals exhibit abnormalities in behaviour and/or locomotor excitation. After ligation of the basilar artery, EEG desynchronization is elicited in rabbits by intravertebral but not by intracarotid injections of α -methyltryptamine [1248].

g) *Effects of α -ethyltryptamine.* In doses of 10 mg/kg, DL- α -ethyltryptamine induces in the cat, within one hour, a 'sympathetic-like' central excitation with a concomitant loss of spontaneous motor activity. Recovery from these effects takes 24 hours. The spinal and primary (somato-sensory) afferent pathways are not affected in doses sufficient to produce gross behaviour changes. The EEG pattern of the 'cerveau isolé' is not altered whereas in the intact animal there is a sustained low voltage fast activity characteristic of arousal. The EEG arousal pattern is accompanied by a behaviour arousal [799b].

h) *Effects of fluorotryptamines.* 0.25 mg of 5-fluorotryptamine injected intracerebrally into mice immediately produces a stuporous state; 1 mg/kg 5-fluorotryptamine and 2 mg/kg 5-fluoro-7-methyltryptamine given to dogs by intraventricular injection produce, like tryptamine, a catatonic state accompanied by defaecation of fluid stools, strong rectal tenesmus and salivation [350].

13.25 Other indolealkylamines

Gramine, 15 mg/kg, injected into the carotid artery causes a significant reduction of the depression produced in the geniculate transmission of the brain by subsequent administration of LSD [387d].

BAS (benzyl dimethylserotonin), 2 mg/kg i.v., induces convulsion-like non-spreading electric discharges in the hippocampus of the rabbit [981].

BAB (benzyl dimethylbufotenine), 10 mg/kg i.v., produces continuous fast activity localized in the lenticular nucleus, chiefly in the putamen. The amplitude is three to four times larger than that of waves of comparable frequency normally present in the lenticular nucleus and in other parts of the rabbit brain [981].

Concerning the central effects of the indolealkylamines other than 5-HT it should be kept in mind that:

- a) tryptamine is a normal constituent of brain tissue.
- b) bufotenine, tryptamine and, probably, also psilocybin, psilocin, dimethyltryptamine, diethyltryptamine and gramine are able, in contrast to 5-HT, to cross the blood brain barrier. Direct evidence of this possibility has been obtained for bufotenine, which could be visualized in chromatograms of brain extracts [386d].
- c) the capacity of some indolealkylamines to cross the blood-brain barrier does not exclude the possibility that part of their central effects may be induced

reflexly through stimulation of peripheral receptors or indirectly through action on the brain vessels.

d) dimethyltryptamines produce central effects which are considerably different from those produced by the primary amines also from a qualitative point of view.

Conclusions concerning the physiological significance of biogenic brain indolealkylamines drawn from the central actions of non-biogenic amines must be clearly labelled as speculation at the present time.

13.3 Biochemical and pharmacological effects of 5-HTP and related precursor aminoacids

13.31 Effects on brain indolealkylamine levels

UDENFRIEND *et al.* [1223, 1227] and DAVIDSON *et al.* [304] first demonstrated that administration of DL-5-HTP produces a conspicuous increase in the 5-HT level of numerous tissues, including brain.

These results have been confirmed and extended by other research workers.

In mice 200 mg/kg 5-HTP given intraperitoneally raises the brain 5-HT level from 0.4 to 1.0 $\mu\text{g/g}$ after 2 hours [304]; in rats 150 mg/kg 5-HTP given intraperitoneally produces an increase of brain 5-HT from 0.4 to 2.4 $\mu\text{g/g}$ after 3 hours [1227], 100 mg/kg subcutaneously from 0.25 to 0.56 $\mu\text{g/g}$ after 1 hour, 100 mg/kg intraperitoneally from 0.32 to 0.88 $\mu\text{g/g}$ after 30 minutes [144, 386]. Similar results have been obtained in other animal species. In rabbits a rise in brain 5-HT from 0.5 to 1.5 $\mu\text{g/g}$ is observed 3 hours after the intraperitoneal administration of 75 mg/kg 5-HTP; in dogs the rise in the amine level is even more conspicuous one hour after 60 mg/kg 5-HTP intravenously: from 1.5 to 13.3 $\mu\text{g/g}$ in the hypothalamus and from 0.2 to 2.4 $\mu\text{g/g}$ in the cortex. The 5-HT concentration increases markedly also in the medulla-pons and in the midbrain; in the cerebellum the increase is much less [277]. Unlike other tissues, the 5-HT level in the brain returns to normal within a few hours [304, 386].

Pretreatment with MAO inhibitors conspicuously enhances the 5-HT accumulation in brain tissue produced by 5-HTP. This enhancing property on the 5-HT accumulation subsequent to 5-HTP administration seems to be shared, to a lesser degree, by diphenylhydantoin [144] and chlorpromazine [277].

The greatest 5-HT concentration following intravenous administration of 5–7 mg/kg 5-HTP to dogs premedicated with 2 mg/kg tranlycypromine was found by HIMWICH and COSTA [580c] in the caudate nucleus, but the amount of this increase did not correlate with the severity of the behavioural symptoms presented by the animals. The pons was the only structure in which the 5-HT concentration could be correlated with behaviour.

Similarly to 5-HTP, L-tryptophan and 4-HTP, which are substrates for brain decarboxylase, evoke an increase or the appearance in the brain of the corresponding amines.

800 mg/kg L-tryptophan given by mouth to guinea-pigs and other animal species increases the brain tryptamine from 0.1 to 1.4 $\mu\text{g/g}$ [574]. However,

neither excess nor deficiency of dietary tryptophan influences brain 5-HT levels clearly [1347, 1168].

100 mg/kg DL-4-HTP, given intraperitoneally to rats, causes the appearance in brain tissue of 1.2–1.5 g μ /g 4-HT [381, 386b].

With 5-hydroxy-N-acetyl-TP and 5-acetoxy-N-acetyl-TP no significant rise in brain 5-HT levels could be demonstrated, although the formation of small amounts of the amine from the precursor aminoacids is highly probable [385].

6-HTP, 7-HTP and 2-HTP (single or repeated intraperitoneal doses of 100 mg/kg) do not affect the 5-HT level in rat brain [386d]. The preliminary finding of COSTA and RINALDI [277] that 5-methyl-TP decreases the 5-HT content in rabbit brain awaits confirmation.

The uptake of 5-HTP by rat brain has been studied *in vitro* by SCHANBERG and GIARMAN [1060]. They have found that slices of brain, as well as of other tissues capable of storing 5-HT and possessing 5-HTP decarboxylase activity, show a facilitated transport of 5-HTP, which is enhanced by oxygen and glucose and is greatly diminished at 0° C. 1 g rat brain is capable of taking up 75 μ g 5-HTP, and more than half the total uptake occurs within 10 minutes.

L-Tryptophan, L-tyrosine and DOPA produce striking inhibition of 5-HTP uptake, whereas phenylalanine, α -methyl DOPA, glutamic acid and γ -aminobutyric acid are without any appreciable influence [1060b].

The above results are strong evidence that the indole aminoacids examined are capable of crossing the blood-brain barrier and of penetrating into the brain tissue. Additional direct evidence has been obtained by paper chromatography of brain extracts. On chromatograms of these extracts the injected aminoacid could be clearly visualized and identified. This is true not only for 5-HTP, 4-HTP, 5-hydroxy-N-acetyl-TP (presence of 5-hydroxy-N-acetyl-TP and 5-HTP), 5-acetoxy-N-acetyl-TP (presence of 5-acetoxy-N-acetyl-TP, 5-hydroxy-N-acetyl-TP and 5-HTP), but also for 7-HTP and 6-HTP [386d].

At this point, however, a big problem arises. Where do indole aminoacids accumulate within the brain tissue, and where are they decarboxylated? It is superfluous to say that the answer to these questions would be of great importance in the interpretation of the central pharmacological effects of 5-HTP and related aminoacids.

13.32 Behaviour and neurological effects

There is general agreement that low doses of 5-HTP (5–20 mg/kg, i.v.) produce in dogs and cats a decrease in spontaneous activity and in rabbits a tranquillizing action on behaviour [141, 840, 735, 278].

Concerning the effect of large doses of 5-HTP, on the contrary, results are partly at variance. UDENFRIEND *et al.* [1223, 1227], BOGDANSKI *et al.* [141], MONNIER [840] and COSTA *et al.* [278] have all found that 5-HTP, given intravenously in doses of 30–100 mg/kg causes in dogs, cats and rabbits excitement and disorientation, accompanied by evidence of depressant effects on certain reflexes (loss of light reflex and of contact plantar reflex), on motor control (tremors, ataxia), and on sensory functions (apparent blindness and unrespon-

siveness to alarming stimuli), as well as by fear in dogs and sham rage in cats. In addition, there is mydriasis, piloerection, sexual excitation, tachycardia, salivation, lacrimation, tachypnoea, retching, vomiting and increased intestinal motility. All the symptoms of 5-HTP are largely prevented by chlorpromazine and enhanced by pretreatment with iproniazid or other MAO inhibitors. The autonomic effects of 5-HTP are generally decreased or prevented by general anaesthesia [278].

Behavioural changes caused by 5-HTP were more severe following injection of the drug into the vertebral artery than after intravenous injection or injection into the carotid artery, indicating that brain areas which normally receive blood from the vertebral artery are more sensitive to 5-HTP than areas which receive blood from the carotid artery. It is suggested that the major site of 5-HTP action lies in some place between the midmedullary level and the area of the brain normally supplied with carotid blood [580c].

According to CRONHEIM and GOURZIS [284b] 5-HTP, while producing hyperexcitability in normal dogs, causes a reinforcement of sedation in reserpinized animals, both when given by infusion and by rapid i.v. injection (30 mg/kg).

In pigeons, administration of 25–75 mg/kg 5-HTP intramuscularly or orally produces a reduction of the pecking response, as measured in a Skinner box. This effect is markedly enhanced by pretreatment with iproniazid and enhancement lasts as long as MAO block endures [28].

To the above clearly positive results other considerably less evident and partly divergent results should be opposed. MALCOLM and LEWIS [735], for example, have been unable to reproduce in cats the symptoms described by BOGDANSKI *et al.* [141]. They could find no signs of central excitation after administration of 5-HTP to unanaesthetized cats, the only sign of excitation being some respiratory stimulation. In particular, they never saw any sign of sham rage in cats after 30 mg/kg 5-HTP or after 100 mg/kg iproniazid followed by 30 mg/kg 5-HTP. The usual effect of 5-HTP was found to be slight depression.

In mice the only appreciable and reproducible effect of 5-HTP given alone may be seen when examining the intraspecific aggressive behaviour of the animals. In fact, at intraperitoneal doses of 100 mg/kg, 5-HTP abolishes fighting in 50% of the treated couples of males. The same effect is obtained with 120 mg/kg 4-HTP [382].

In rats 5-HTP alone fails to produce distinct central effects, the only ambiguous central symptoms being lethargy and a relative lack of response to stimuli, even when administered intraperitoneally every 12 hours for periods up to 4 months, in doses of 200 mg/kg.

Pretreatment with MAO inhibitors was found to be necessary in mice and rats before 5-HTP and 4-HTP could produce tremors, excitement or increased spontaneous activity [141, 386].

According to BROWN [179b], not only 5-HT and 5-HTP but also L-tryptophan is in mice a powerful depressant both of spontaneous activity and of hyperactivity produced by cocaine or amphetamine. At a 50% depression level

of spontaneous activity, L-tryptophan is some 60 times more potent than either 5-HT and 5-HTP. However, the effect of tryptophan does not increase as the dose is increased.

13.33 Potentiation of barbiturate hypnosis

Hexobarbital and chloral hydrate anaesthesia is prolonged in mice by 5-HTP (15–40 mg/kg, i.v.) and, surprisingly enough, also by tryptophan (20–600 mg/kg) which, in some experiments, is at least as effective as 5-HTP. In mice pretreated with iproniazid, 5-HTP and tryptophan produce central excitation which prevents the narcotic action of hexobarbital from appearing [593, 730].

13.34 Pyretogenic effect

5-HTP administered to rabbits produces a pyretogenic response which is roughly proportional to the dose administered, and varies in intensity according to the size of the animal. In animals weighing 2 kg, the minimum active dose is approximately 25 mg/kg, i.v.; with 75 mg/kg there is a rise of rectal temperature by 4° C and death occurs at the peak of the hyperpyrexia. Iproniazid (50–100 mg/kg) and β -phenylisopropylhydrazine potentiate this response markedly (minimum effective dose of 5-HTP: 2–5 mg/kg). Both the LSD and 5-HTP pyretogenic effects are greatly attenuated by prior administration of BOL, and, after tolerance has developed in rabbits by daily injections of progressively increasing doses of LSD, the usual 5-HTP pyretogenic response is greatly diminished, presumably because of a cross tolerance phenomenon [597, 598].

4-HTP apparently displays a more intense pyretogenic effect than 5-HTP itself. In fact, in rabbits pretreated with iproniazid, intravenous doses of 5 mg/kg 4-HTP produce an increase in rectal temperature (1.5–2° C) similar to that caused by 15 mg/kg 5-HT [382].

Mice are less sensitive than rabbits to the pyretogenic effect of 5-HTP and 4-HTP. However, in animals pretreated with iproniazid both aminoacids produce a rise in rectal temperature, which is 0.5° C for 30 mg/kg, and 1.5° C for 60 mg/kg, intraperitoneally.

13.35 Reversal of reserpine effects

In mice pretreated with 20–40 mg/kg reserpine intraperitoneally, 5-HTP is unable to antagonize the effects of the tranquillizing drug, when given in intraperitoneal doses up to 1000 mg/kg. However a mixture of equal amounts of 5-HTP and DOPA (250–500 mg/kg) produces a more complete and longer-lasting reversal of the reserpine effects than does the same dose of DOPA alone [222].

The antagonizing effect of 5-HTP (100–300 mg/kg) is apparently more evident in rabbits and rats pretreated with small doses of reserpine (2 mg/kg) [141].

13.36 Action on conditioned escape reflexes

5-HTP (100 mg/kg i.p.) produces in rats a moderate inhibition of escape reaction, lasting a few hours. This effect is enhanced by harmaline (5–10 mg/kg i.p.) [1175].

13.37 Action on brain electrical activity

COSTA and RINALDI [277] have observed that the intravenous injection of 75 mg/kg 5-HTP produces in rabbits an EEG pattern characterized by mono-rhythmic diffuse high voltage activity, with disappearance of cortical fast rhythms and hippocampal theta waves, followed by a generalized depression of voltage.

Slow intracarotid injection of 22 or 44 mg 5-HTP into intact curarized rabbits has been found by COSTA *et al.* [280b] to cause a biphasic variation of synchronized and desynchronized EEG patterns of the brain. Synchronization is regularly observed in the first 20–30 minutes, and is followed by re-establishment of the control pattern in all animals given 22 mg 5-HTP and in 50% of those given 44 mg. In the remaining 50% of rabbits, more precisely in those showing a 3-fold increase of midbrain 5-HT content, synchronization is followed by enduring desynchronization.

Working on the same experimental animal MONNIER *et al.* [840, 855, 842] have obtained a very complicated EEG response with 5-HTP. Following small intravenous doses of the aminoacid (10–20 mg/kg) the spontaneous electrical activity of the neocortex shows bursts of slow rhythms for 30 minutes, then a desynchronization, whereas the paleocortex and the diencephalon present a mixture of slow activities and of feebly synchronized or nearly desynchronized activities. Excitability of the reticular system decreases, that of the medio-thalamic system increases. Later on, these manifestations give place to an EEG syndrome of moderate arousal with desynchronization of the neocortex and intermittent synchronization of the paleocortex and the diencephalon.

Large intravenous doses of 5-HTP (50–85 mg/kg) produce a considerably different picture, characterized by polyphasic alterations of the electrical brain activity. A first period of slow high voltage activity is soon followed by a generalized desynchronization of the neocortex, the paleocortex and the meso-diencephalon. Desynchronization persists after mesencephalic section. MONNIER *et al.* suggest that the observed activation syndrome depends mainly upon activation of the hippocampic paleocortex and, to a lesser degree, upon inhibition of the mediotthalamic recruiting system.

The main changes in the electrical activity of the rabbit brain observed by DOMER and LONGO [332b] following repeated intravenous injection or intravenous infusion of 20–100 mg/kg 5-HTP consist of flattening of the electrocorticogram and development of 30–40 c/sec discharge in the rhinencephalon. Premedication with α -methyl DOPA (100 mg/kg s.c., and 50 mg/kg, i.p.) or with chlorpromazine (5 mg/kg) delays the onset of the 5-HTP effects, while pre-medication with β -phenylisopropylhydrazine, reserpine or atropine does not affect the onset or full development of the rhinencephalic discharge. After the

discharge activity is fully developed, it is greatly depressed or abolished by administration of pentobarbital or mephensin.

Contrary to the preceding groups of research workers, GLÄSSER and MANTEGAZZINI [493] carried out their experiments in the cat, employing two preparations, the first with a spontaneously desynchronized EEG, and the second with a spontaneously synchronized EEG. 5-HTP (8–60 mg) was administered by slow intracarotid injection. The main results were as follows:

a) In the midpontine pretrigeminal preparation, 8–20 mg 5-HTP produces a synchronization of the spontaneously activated EEG quite similar to that caused by small doses of thiopental. The effect starts 1–3 minutes after injection and lasts 30–45 minutes. Olfactory and visual stimuli generally provoke a clear arousal reaction. The action of 5-HTP becomes more intense by increasing the dose (40–60 mg) and the arousal reaction produced by sensory stimuli is often abolished.

b) When the experiment is arranged in such a way that blood from the two carotids prevalently irrigates the corresponding hemispheres, 5-HTP produces an asymmetric EEG deactivation which is more evident in the hemisphere ipsilateral to the injected carotid.

c) In the midpontine pretrigeminal preparation, completed with mesencephalic hemisection, 5-HTP enhances the spontaneous synchronization proper to this preparation.

REVZIN and COSTA [979] have observed in unanaesthetized cats a depressant effect of 5-HTP (100–200 mg/kg i.v.) on the potentials evoked in the hippocampus by stimulation of the amygdala. The depression of potentials is slight with 5-HTP alone but considerably more intense after premedication with the MAO inhibitor tranlycypromine.

From the above, it may be seen that the results obtained by the various investigators are at partial variance, depending probably upon the experimental procedure and the animal species. It is, of course, very difficult to decide whether one experimental method or one animal species is preferable to another.

However, from a general point of view, it may be stated:

α) That close intraarterial injection gives more reliable results than systemic injection, because the first route of administration largely avoids the cause of error represented by the possible interference with the true central effects of the 5-HT generated by the precursor aminoacid within the CNS, of the peripheral effects produced by the 5-HT generated outside the CNS.

A brilliant demonstration of the validity of this statement has been given by GLÄSSER and MANTEGAZZINI, who succeeded in provoking with 5-HTP an asymmetric synchronization of EEG ipsilateral to the injected artery.

β) That it is questionable whether the rabbit can be considered the animal of choice for studying the EEG effects of 5-HTP. In fact, it is well known that precisely in the rabbit 5-HTP produces a pyretogenic effect of exceptional intensity (death may occur at the peak of temperature rise), presumably capable, by itself, of altering the electrical activity of the brain, especially in the waking animal.

In the opinion of the majority of the research workers 5-HTP acts centrally only through the 5-HT originating from it, and it is generally assumed that the 5-HT which derives from 5-HTP produces the same effects as strictly endogenous brain 5-HT. These views should be accepted with caution.

First of all, it is by no means demonstrated that endogenous 5-HT has the same localization and possesses the same actions as the 5-HT generated from 5-HTP. In fact, exogenous 5-HTP may be totally or in part decarboxylated in cells different from those which decarboxylate endogenous 5-HTP, and it is obvious that the effects elicited by the 5-HT originating from the two different sources may be different. PROCKOP *et al.* [960], for example, found that whereas MAO inhibitors protect mice and rats against electroshock and metrazol convulsant seizures, both 5-HTP and DOPA are completely ineffective. If MAO inhibitors display their protective action by preserving endogenous amines from inactivation, it should be concluded that these amines act in a different way or on different receptors from amines originating from exogenous 5-HTP or DOPA.

Secondly, it should not be forgotten that the enzyme acting on 5-HTP to produce 5-HT is by no means specific for 5-HTP. DOPA and other aromatic aminoacids are also excellent substrates for it. Under these circumstances it would be quite possible for the relatively large doses of 5-HTP necessary to produce pharmacological effects to hinder the decarboxylation of endogenous DOPA and/or other aminoacids a) through saturation by the excess 5-HTP of the decarboxylating capacity of the brain enzyme and b) through a more direct inhibition by 5-HTP of DOPA decarboxylation [1338].

In both cases excessive 5-HT production would be accompanied by deficient catecholamine biosynthesis, with the consequent rupture of the equilibrium existing between the various brain amines and the consequent possibility that 5-HTP displays its central effects not only through hyperproduction of 5-HT but also through lack of catecholamines and other amines as well.

Thus, contrary to expectation, even the use of 5-HTP does not permit any definite and unambiguous conclusion to be drawn about the function of endogenous brain 5-HT.

13.4 Effects of monoamine oxidase inhibitors

13.41 Effects on endogenous brain 5-HT

Oral or parenteral administration of MAO inhibitors regularly produces in all experimental animals examined a conspicuous increase in the brain 5-HT level.

This is of the order of 25–150% after a single dose, but reaches 300–400% and even 600–700% after repeated administration [935, 1230, 938, 1187, 254]. For example, rats kept on a diet containing 0.35% iproniazid, show, after a week, an increase of brain 5-HT from the initial 0.5 μg to 2.6 $\mu\text{g/g}$ [93]. Similarly, in dogs and cats given daily subcutaneous doses of 2 mg/kg β -phenylisopropylhydrazine, the brain 5-HT level increases from 0.75 to 5.05 $\mu\text{g/g}$ within 12 days

(dogs) and from 0.7 to 2.9 $\mu\text{g/g}$ within 7 days (cats) [175]. It is of interest that repeated, large doses (2–4 mg/kg) of β -phenylisopropylhydrazine markedly increase the 5-HT content in the spinal cord, too, the level becoming especially high (1.5 $\mu\text{g/g}$) in the lumbar region [1158].

Concerning the action of MAO-inhibitors on free and bound brain 5-HT (the first contained in the supernatant, the second in the particulate fraction of ultracentrifuged brain homogenates), it has been found that iproniazid causes in the rat brain a rise in the 5-HT content of both fractions, but produces a shift in the bound-free ratio from 6–3.5 to 1.5–1.6, i.e. it produces a considerably greater elevation of free 5-HT.

Within 72 hours the bound 5-HT returns to control levels, while the free 5-HT is still about 50% higher than controls. Iproniazid pretreatment inhibits the release of 5-HT elicited by reserpine [481, 500].

Striking differences could be observed among the various MAO inhibitors not only in regard to their relative potency, but also in the rapidity of onset and the duration of their effect on brain 5-HT levels.

Iproniazid, as a typical representative of the slow-acting, long-lasting MAO inhibitors, causes a relatively slow rise in the brain 5-HT content, which is hardly visible after 2 hours [1111], reaches its maximum after 6–8 hours, and lasts several days [935, 938, 880, 175]. β -Phenylisopropylhydrazine, a representative of the rapid-acting, long-lasting MAO inhibitors produces an almost immediate rise of brain 5-HT (50% increase within 10–15 minutes in rabbits, and within 50 minutes in mice) which then persists for 12–15 days [1157, 254, 174].

Finally, harmine and harmaline, representatives of the rapid-acting, short-lasting MAO inhibitors, act even more promptly than β -phenylisopropylhydrazine in producing an increase of brain 5-HT (in the rat: from 0.4–0.45 μg to 0.7–0.75 $\mu\text{g/g}$ within 10 minutes), but their effects wear off within 6 hours [1230].

From the experiments of CHESSIN *et al.* [254] it seems conceivable that more than 50% inhibition of MAO is required in order to elevate the brain 5-HT significantly. According to GEY and PLETSCHER [475b], MAO block should be even more complete (85–95%).

The reason for the different rates of action of the various MAO inhibitors is not clear. GOGERTY and HORITA [502] found that brain MAO activity is depressed more promptly by β -phenylisopropylhydrazine than by iproniazid. BRODIE *et al.* [175] on the contrary, affirm that a rapid abolition of MAO activity is observed in the brain of animals given either iproniazid or β -phenylisopropylhydrazine. These findings would suggest that both drugs readily penetrate the brain, but that β -phenylisopropylhydrazine reaches the actual site of MAO activity at a faster rate than iproniazid.

PLETSCHER *et al.* [947] observed that pretreatment with harmaline hinders in rats and mice the increase of brain 5-HT levels produced by iproniazid or other long-lasting MAO inhibitors. However, harmaline is ineffective when given 6–8 hours after iproniazid.

The suggested explanation is that the short-acting harmaline blocks the receptors which then can no longer be attacked by the long-acting hydrazides.

Harmaline, however, would not be capable of reversing the very strong hydrazide-enzyme bond once it had taken place.

Similarly to harmaline, chlorpromazine, too, counteracts the increase in brain 5-HT and noradrenaline caused by iproniazid, when given prior to this drug in intraperitoneal doses of 20 mg/kg [949, 352b]. Chlorpromazine does not possess this effect in guinea-pigs and rabbits, nor does it affect the normal content of 5-HT as well as the 5-HT increase in rat brain produced by administration of 5-HTP. It is assumed that chlorpromazine decreases the permeability of the storage depots for amines [949].

In the case of rapid-acting MAO inhibitors the rate of 5-HT accumulation does not appear to be increased by increasing the dose over an optimum level, which is probably the one which rapidly produces a 100% inhibition of MAO. On the other hand, for all the long-lasting inhibitors, the ultimate level of 5-HT achieved is determined by the size of the dose. For example, 48 mg/kg β -phenylisopropylhydrazine produces in mice an increase in brain 5-HT which is 3 times as great as that produced by 12 mg/kg [254].

It is of great interest, even from a therapeutic point of view, that small repeated doses of long-lasting MAO inhibitors are capable of provoking an increase in the brain 5-HT level as great as that caused by a single large dose. For example, the daily administration of as little as 0.1 mg/kg β -phenylisopropylhydrazine elicits, after 15 days, a two- to three-fold increase of 5-HT in the dog brain [1158]. Evidently, the successive doses join the preceding ones, owing to the existence of a very strong inhibitor-enzyme bond.

Pretreatment with 100 mg/kg desoxyipyridoxine or decreasing body temperature by chilling causes, both in normal mice and in those pretreated with reserpine, a slowing down in the rate of increase of brain 5-HT produced by β -phenylethylhydrazine [340, 342].

Rats, mice and rabbits pretreated with iproniazid and then given reserpine or tetrabenazine show a brain 5-HT content which is lower than that presented by animals given iproniazid alone, but considerably higher than that presented by animals given reserpine alone. In fact, the 5-HT level never falls below the control values [683]. In mice pretreated with iproniazid, 20 mg/kg reserpine has a 5-HT-depleting effect less intense than that possessed by 2 mg in normal animals; and after the injection of Catron®, 5-HT continues to be formed at a near-normal rate in the brain of reserpine-treated mice whose body temperatures are maintained at approximately normal levels [342]. Moreover, the brain 5-HT and catecholamine content depressed by previous injections of reserpine, display enhanced recovery if iproniazid is administered 8 hours after reserpine [935, 936, 938].

As with 5-HT, tryptamine, which is the other indolealkylamine normally occurring in brain tissue, is influenced by MAO inhibitors. In fact, pretreatment of guinea-pigs with β -phenylisopropylhydrazine or iproniazid produces an increase in brain tryptamine level from the normal 0.1 to 0.3–0.7 $\mu\text{g/g}$ [574].

The divergent, negative results obtained in rats by GREEN and SAWYER [519] may perhaps depend upon the exceedingly low dosage of MAO inhibitors.

MAO inhibitors act not only on the 5-HT level in the brain of mammals, but they also decidedly increase the 5-HT content in ganglia of invertebrates. Following administration of harmaline or β -isopropylphenylhydrazine the 5-HT concentration in the optic ganglia of *Eledone* rises from the normal 2–4 to 4–8 $\mu\text{g/g}$ [98].

13.42 Effects on brain amines originating from exogenous precursor aminoacids

There is striking evidence that MAO inhibitors enhance the 5-HT accumulation in brain tissue produced by the administration of 5-HTP.

The following examples may be quoted:

	Brain 5-HT ($\mu\text{g/g}$)			
	Controls	Iproniazid 100 mg/kg 12–20 hr before	5-HTP 70–100 mg/kg 2 hr before	2 + 3
Rabbit [1227]	0.5	0.8	1.5	3.8
Rat [1227]	0.63	1.1	1.3	4.1
Rat [935, 938]	0.5–0.65	0.9–1.3	0.65–0.85	2.2–3.3
Rat [386]	0.46	0.74	0.78	1.68

As with 5-HTP, other precursor aminoacids produce a more marked elevation of the corresponding amines when they are administered after pretreatment with iproniazid. This is true for L-tryptophan [574], N-acetyl-5-HTP, N-acetyl-5-acetoxy-TP and 4-HTP [385, 386b, 386d].

The amine values in control rats, in those pretreated with iproniazid alone, in those given the aminoacid alone, and, finally, in those given iproniazid plus aminoacid are, respectively, as follows:

5-HT for N-acetyl-5-HTP: 0.53, 0.81, 0.55 and 1.07 $\mu\text{g/g}$.

5-HT for 5-acetoxy-N-acetyl-TP: 0.53, 0.81, 0.47 and 0.99 $\mu\text{g/g}$.

4-HT for 4-HTP: 0 0 1.3 and 2.1 $\mu\text{g/g}$.

13.43 Potentiation of the central effects of 5-HT and 5-HTP

5-HT given intravenously to iproniazid pretreated mice fails to cause excitation, but intracerebral 5-HT does produce CNS stimulation, as shown by increased motor activity. The minimum effective dose of 5-HT is 1 μg [252, 253].

Whereas the potentiating effect of 5-HT on ethanol narcosis is not significantly influenced by premedication with iproniazid [105], prolongation of barbiturate anaesthesia by 5-HT and tryptamine is potentiated in mice by iproniazid [593].

Similarly, iproniazid and methylphenethylhydrazine enhance convulsions induced in rats by intravenous injections of tryptamine [1192, 1194].

However, the most striking consequences of MAO block are observed in regard to the central effects of 5-HTP.

In fact, both the excitatory and the pyretogenic effects of 5-HTP are strongly potentiated by these drugs [1227, 597, 598]. After 5 mg/kg β -phenylisopropylhydrazine, the minimum dose of 5-HTP required to produce lethal hyperpyrexia falls from 75 mg/kg to 10 mg/kg, and the minimum dose active on temperature decreases from 25–50 mg to less than 5 mg/kg. In rats and mice, pretreatment with a MAO inhibitor is actually required before 5-HTP produces its characteristic effects. One of these effects, the tremors, has been suggested by LESSIN [726] as a criterion for the pharmacological evaluation of MAO inhibitors.

It is of interest to note that whereas iproniazid and 5-HTP *per se* are ineffective or nearly so, the combination of the two drugs elevates the threshold dose of metrazol necessary to produce convulsions in mice [665] and prolongs the survival time of mice infused with metrazol [725]. PROCKOP *et al.* [960] also have obtained a distinct anticonvulsant effect of 5-HTP in rats pretreated with iproniazid.

Starting with the observation that after iproniazid pretreatment 5-HTP (15–40 mg/kg) potentiates morphine analgesia in rats [1062], it has been suggested that 5-HT plays a role in the analgesic action of morphine.

13.44 Some central effects proper to MAO-inhibitors

Since it is by no means demonstrated that the central effects of MAO-inhibitors can be correlated with interferences in brain 5-HT metabolism, we shall limit ourselves to summarizing some essential points, referring to the monograph by PLETSCHER *et al.* [948] for details.

a) Large lethal or sublethal doses of MAO-inhibitors produce in all species examined (mice, rats, rabbits, cats) excitation and other behavioural changes, accompanied by signs of autonomic nervous system stimulation [354]. However, single lower doses, capable of giving maximum MAO-inhibition and of producing enormous increases in brain 5-HT levels, fail to produce obvious central effects. These make their appearance in rabbits, but not in cats or dogs, only after repeated administration of MAO-inhibitors [157, 1156, 1155].

b) MAO-inhibitors prolong barbiturate sleeping time [1072, 1009, 436b].

c) MAO-inhibitors provok a reversal of the effects of reserpine. In fact, animals pretreated with MAO-inhibitors and then given reserpine, present, instead of the usual sedation typical of reserpine alone, an intense excitation with sympathetic stimulation [105, 252, 1192, 1157, 388c]. This 'reserpine reversal' is seen in mice, rats, guinea-pigs, rabbits, cats, dogs and monkeys. If rabbits are given reserpine and then β -phenylisopropylamine, sedation, miosis and relaxation of the nictitating membrane is abolished within 30 minutes [1157]. Pretreatment with iproniazid also reduces the potentiating effect of reserpine on ethanol narcosis [105], prevents reserpine hypothermia [305, 726, 46], markedly reduces mortality produced in mice by reserpine [252] and protects mice against reserpine facilitation of metrazol convulsions [1009, 567] or even reverses the effects of reserpine on electroshock convulsions [959].

'Reserpine reversal' cannot be observed in human patients, at the clinically effective dose levels of MAO-inhibitors and reserpine [977b].

d) According to PROCKOP *et al.* [960, 961, 959] both irreversible and reversible MAO-inhibitors prevent in rats tonic extension of the hind limbs produced by supramaximum electroshock. In addition, β -phenylisopropylhydrazine blocks in mice the tonic extensor phase and decreases mortality produced by intravenous metrazol.

These results have not been confirmed generally. In fact, of 25 MAO-inhibitors tested in mice at 1-, 4- and 24-hour intervals, using 'psychomotor', supramaximum electroshock and metrazol tests, none has shown anticonvulsant activity and several of them proved to be ineffective against tremorine tremor [388c]. Similarly, KOBINGER [665] and HERTTING [567] have failed to find any influence of iproniazid on the threshold doses of metrazol producing tonic extension cramps in mice and on the doses causing tonic lethal cramps.

While corroborating the positive results of PROCKOP *et al.* [960], CHOW and HENDLEY [255] do not believe that the weak anticonvulsant activity of MAO-inhibitors is directly correlated with MAO-inhibition *per se*.

13.45 Discussion

Treatment with MAO-inhibitors produces in all animal species examined a more or less conspicuous and prolonged increase in the brain level of endogenous 5-HT, and it greatly potentiates the increase in brain indolealkylamines produced by administration of precursor aminoacids. MAO-inhibitors have also been very useful in determining the turnover rate of 5-HT and tryptamine in brain tissue, and they have been of decisive importance in trapping amines, which were undetectable or hardly detectable without MAO-blockade.

These are uncontroversial facts and on these points there is full accordance of opinion. However, opinions become largely divergent when trying to interpret the pharmacological actions of MAO-inhibitors.

The first question to be solved in this respect is that of whether the potentiation by MAO-inhibitors of the pharmacological effects produced by 5-HTP should be ascribed solely to the much greater accumulation of 5-HT obtained by the combination of MAO-blockade and administration of the precursor aminoacid. Whereas in some cases the answer should be positive (e.g. potentiating effect of MAO-inhibitors on the pyretogenic response to 5-HTP), in others it may be dubious. For example, it is rather surprising that in rats and mice neither 5-HTP (and 4-HTP) alone nor MAO-inhibitors alone produce any sign of excitation and that the administration of both drugs is necessary for the appearance of central stimulation.

It will be seen at once that MAO-inhibitors do not only block MAO and that the brain contains numerous biogenic amines which can be acted upon by MAO. It is therefore conceivable that if 5-HTP becomes active in rats and mice only following pretreatment with MAO-inhibitors this is not due simply to the greater accumulation of 5-HT obtained in this way, but also to the fact that

5-HT acts on a substratum profoundly changed in regard to enzyme activity and to concentration in other amines.

A second, even more important question to be posed is the following: are the central effects produced in experimental animals and, still more, in humans, by MAO-inhibitors administered alone causally related to the increase in brain amines? [1156]. More precisely, is there any connection between the central pharmacological effects of these drugs and the accumulation of 5-HT and nor-adrenaline [1155]? Much water has been poured on the fire of the first enthusiastic reports and conclusions. At the beginning of the story of iproniazid and other MAO-inhibitors everything they did was ascribed to 5-HT. Then catecholamines dethroned 5-HT even in the minds of some of the more convinced upholders of the supremacy of 5-HT [1160]. At the present time, a prudent position of expectation is taken up by an increasing number of investigators.

A few considerations fully justify this attitude:

a) MAO-inhibition is by no means the only action of the so-called MAO-inhibitors. As research progresses, the list of enzymes acted upon by these drugs becomes longer, while at the same time the interpretation of their mechanism of action grows more obscure.

Iproniazid and other MAO-inhibitors, besides MAO, also depress to a greater or lesser degree diamineoxidase [192, 263, 129, 743, 1115b], pyridoxal phosphokinase [806, 966], succinic dehydrogenase [966], α -glycerophosphate dehydrogenase [973b], ceruloplasmin [1344], DOPA decarboxylase [341, 515], L-glutamic acid decarboxylase [984, 1123] and other B₆-containing enzyme systems, through their capacity to enhance urinary B₆ excretion and to produce a pyridoxine deficiency [402, 956]. In addition, MAO-inhibitors increase the rate of destruction of adrenochrome in blood [586] and interfere with some basic steps of intermediary oxidative metabolism [669, 464] as well as with the diphosphoridine nucleotide metabolism [670b].

However, it has been claimed that there are considerable differences among the various MAO-inhibitors in their action on enzyme systems other than MAO. Nialamide, for example, seems to have little or no effect on DOPA decarboxylase, transaminase or carbonic anhydrase [415], and MAO-inhibitors not possessing a hydrazine moiety do not seem to inhibit diamineoxidase [263] nor dehydrogenases [973b].

b) Many pharmacological and toxic actions of MAO-inhibitors probably have little to do with MAO-blockade. The acute toxic effects of MAO-inhibitors which last a few hours and obviously are nothing but an exaggeration of pharmacological effects, are apparently not related to the MAO-inhibition which persists for several days [388c]; the analgesic activity of MAO-inhibitors is not dependent upon the accumulation of 5-HT or catecholamines [357]; prolongation of hexobarbital sleeping time by MAO-inhibitors is not correlated with any MAO-blockade [354, 1009], but simply with an inhibition of the metabolism of the barbiturate in the liver [436b]; finally, single large doses of iproniazid evoke EEG alerting without any associated changes in 5-HT or catecholamines

[580b] and produce blood pressure changes and cardiac effects which are probably not related to MAO-inhibition [254, 354, 732b].

In a recent critical comment, ROSENBLUM and FERGUSON [997b] affirm that it remains to be demonstrated if MAO-inhibition is directly or indirectly involved in the action of MAO-inhibitors or if the enzyme-inhibiting activity of such agents is merely an unrelated coincidence. A similar concept is shared by SCHERBEL [1067b].

c) Finally, it should always be kept in mind that administration of MAO-inhibitors produces an accumulation in brain tissue of all kinds of amines which can be acted upon by MAO [1233b], and may also cause a remarkable increase of some minor metabolites originating through the attack of the amines by enzyme systems other than MAO [946].

Among the known amines are 5-HT, tryptamine, dopamine, adrenaline, noradrenaline, *m*-O-methyl-noradrenaline, *p*-tyramine, histamine, and possibly *o*-tyramine, *m*-tyramine, phenylethylamine [618b] and 5-methoxytryptamine.

This provisional list is certainly destined to grow longer as amine detection methods become more sensitive and specific. HONEGGER and HONEGGER [595], for example, have recently described the occurrence in brain, in amounts corresponding to those of the other known amines (0.01–2.46 $\mu\text{g/g}$) of methylamine, dimethylamine, ethylamine, pyrrolidine, piperidine and dimethylaminoethanol. It is probable that many amines have so far escaped our observation simply because they do not present the striking peripheral pharmacological effects and the brilliant colour and fluorescence reactions shown by the indolealkylamines and the catecholamines.

To conclude, MAO-inhibitors have contributed enormously, and they will also do so in the future, to the biochemical detection of amines. However, whether they have substantially aided the understanding of the biological significance of these amines is doubtful.

13.5 Effects of DOPA decarboxylase inhibitors

α -methyl DOPA (100 mg/kg, i.m.) prevents in mice pretreated with iproniazid the central excitation produced by 5-HTP (20 mg/kg: i.v.) and removes the antagonistic action of 5-HTP towards iproniazid potentiation of barbiturate hypnosis [1306].

Single doses of α -methyl DOPA (100 mg/kg s.c.) in mice greatly inhibit brain and intestinal decarboxylase. This inhibition reaches its maximum at 30–60 minutes after administration and disappears after 8 hours. Brain 5-HT concentration is reduced to 70% of controls at 60 minutes, thereafter returning to the control value within 8 hours.

Repeated large doses of α -methyl DOPA (400 mg/kg) given to mice at 4-hour intervals reduce brain 5-HT levels to 50% and to 40% of controls, at 12 and 24 hours, respectively. During treatment the animals become very drowsy and still but they are easily aroused on being disturbed [1136].

In guinea-pigs the reduction of brain 5-HT is even more important, reaching 90%.

The above observations of SMITH [1136, 1137] have been fully confirmed by other research workers. GOLDBERG *et al.* [504b] found that single intravenous injections of 200–500 mg/kg of α -methylDOPA or α -methyl-*m*-tyrosine produce in dogs a decrease in the 5-HT content of the caudate nuclei, which is 60% after 3 hours but only 20% after 6 hours, and a concomitant smaller decrease (25–40%) of noradrenaline levels in the atrium. The main pharmacological effects elicited by the DOPA decarboxylase inhibitors are a lowering of blood pressure which begins 2–3 hours after drug administration and reaches a maximum after 4–6 hours, and a concomitant sedation. Cardiac effects elicited by stimulation of the presynaptic fibres to the stellate ganglion remain unchanged, while the inotropic effect produced by intravenous DOPA or *m*-tyrosine is blocked for 30 minutes, then returns to normal within 4–6 hours.

PORTER *et al.* [953b] and HESS *et al.* [574b] have observed that both α -methylDOPA and α -methyl-*m*-tyrosine produce in guinea-pigs and mice depletion of brain 5-HT and catecholamines. Whereas 5-HT depletion lasts only 8–10 hours and approximates the decarboxylase inhibition, depletion of noradrenaline persists for days, long after the enzyme activity has returned to normal levels. On brain amines α -methyl-*m*-tyrosine is approximately twice as active as α -methylDOPA (ED 50 in mice 24 mg/kg versus 65 mg/kg).

HESS *et al.* [574c] have recently demonstrated that the temporal difference between the relatively prompt return to normal levels of 5-HT and the depressed levels of norepinephrine, maintained for days longer, is not due to an inhibition of biosynthesis of norepinephrine but, more likely, to a long-lasting inhibitory effect of α -methylaminoacids on norepinephrine binding sites. In fact, following administration of α -methylaminoacids brain dopamine returns, in sharp contrast to norepinephrine, to normal levels already in 24 hours, thus following closely the pattern characteristic for brain 5-HT, and, on the other side, no disturbance is produced, either *in vivo* or *in vitro*, by α -methyl-*m*-tyrosine in the functioning of dopamine- β -oxidase, the enzyme transforming dopamine into norepinephrine.

On the basis of the similarities existing in many respects between the appearance of animals treated with reserpine and with α -methylDOPA, SMITH suggested that the sedative effects of both drugs might be due to the reduction in brain 5-HT concentration that they produce. This suggestion should be dropped since the main piece of evidence in its favour, the alleged lack of effect of α -methylDOPA on brain noradrenaline, has not been confirmed. At present the depletion of catecholamines, rather than of 5-HT, seems to play a major role in the production of the central and peripheral effects of DOPA decarboxylase inhibitors.

13.6 Effects of reserpine and other 5-HT releasers

13.61 Action on endogenous brain 5-HT

BRODIE *et al.* [167, 932, 933] first demonstrated that reserpine produces a rapid and nearly complete release of the 5-HT contained in brain tissue.

Following the intravenous injection of 5 mg/kg reserpine, the 5-HT level of the rabbit brain, normally 0.55 $\mu\text{g/g}$, declines by about 75% within 30 minutes, and within 4 hours it declines by about 90% (0.05 $\mu\text{g/g}$). It remains at this low level for approximately 24 hours and then increases slowly, attaining the normal value after about seven days. 1 mg/kg reserpine produces the same effect as 5 mg/kg, and single doses of the alkaloid as low as 0.1 mg liberate measurable amounts of 5-HT. The effects of reserpine are the same on different parts of the brain, independently of their 5-HT content. Already from the first experiments it became obvious that in the brain 5-HT is released much more rapidly than in other tissues.

The above fundamental data were soon confirmed and extended by the BRODIE's group and by numerous other investigators. Thus, reserpine has been found to liberate 5-HT from the brain of the dog [876], cat, guinea-pig [1353, 1102, 1269], rat and mouse [366, 594, 66], as well as from ganglia and peripheral nerves of molluscs [919, 98]. Completeness and rapidity of liberation, however, varies from one animal species to another. Rats and mice seem to be particularly resistant to reserpine: in fact, the 5-HT level in rat brain declines only by 40 to 70% after administration of 2 mg/kg reserpine [366, 594, 66].

The intimate mechanism of the reserpine action on brain 5-HT is poorly understood, and studies on centrifuged brain homogenates aimed at clarifying this essential point have produced partly discordant results [746, 500, 481].

All investigators agree that reserpine produces a decrease in both bound 5-HT (contained in the particulate fraction) and free 5-HT (present in the supernatant), but whereas GIARMAN and SCHANBERG [481] and GLUCKMANN [500] have observed a much greater depletion of the particulate store of 5-HT (from 0.38 to 0.06 $\mu\text{g/g}$) than of the 5-HT present in the non-particulate fraction (from 0.063 to 0.032 $\mu\text{g/g}$), exactly the opposite has been noted by LIPTON and GORDON [746].

The observations of WALASZEK and ABOOD [1273] on the *in vitro* action of reserpine on mitochondrial 5-HT are also interesting. These investigators found that whereas reserpine 10^{-6} M completely depletes mitochondria of 5-HT, it does not alter their ability to reabsorb 5-HT from the suspending medium. In fact, when either untreated or reserpine-treated mitochondria are exposed to 5-HT, the 5-HT content increases from 0.02 $\mu\text{g/mg}$ mitochondrial N to a maximum value of 0.06 $\mu\text{g/mg}$. These results are valid for dopamine also: in *in vivo* experiments, WEIL, MALHERBE and BONE [1282] demonstrated that reserpine does not prevent the incorporation and retention in bound form of the amine newly formed from DOPA.

Thus, from the data available at the present time it may be said that reserpine impairs 5-HT binding sites within the brain, so that they lose, in part, their capacity to retain 5-HT. The 5-HT liberated from its granular stores does not accumulate in the cells but is rapidly eliminated or destroyed [682]. If results obtained outside the CNS are transferable to the brain, it may also be stated that reserpine does not interfere in the biosynthesis of 5-HT.

Besides reserpine, other natural and semi-synthetic reserpine-like alkaloids, all possessing tranquillizing action, release 5-HT from brain tissue. This is true

for deserpidine, rescinamine (very active on the rabbit brain, but much less so on the mouse brain) [171, 172], raunescine [879, 630], carbethoxy-syrinogoyl-methylreserpate or SU 3118, and, to a lesser degree, for methyl-o(3-dimethylaminobenzyl)reserpate or SU 5171 [413].

Other *Rauwolfia* alkaloids, devoid of sedative action, do not affect the brain 5-HT concentration. This is the case with isoreserpine, isoraunescine, methylreserpate, reserpic acid, reserpinine, serpentine and others [879, 413].

Reserpine and related alkaloids are not the only compounds capable of releasing brain 5-HT. Other 5-HT releasers, belonging to different chemical classes have been described and it is quite probable that the list of these substances will lengthen.

So far, the most interesting series of compounds has been that of benzoquinolizine derivatives with tetrabenazine being the most potent of them: its 5-HT depleting effect is 4 times less intense than that of reserpine in mice, 10 times less intense in rats [937] and 20 times less intense in rabbits [940].

Maximum brain 5-HT depletion following tetrabenazine administration (40 mg/kg) is attained in rats after 30 minutes and it is never more than 65–75%. Return to control values takes only 10–24 hours [937]. 3,4,5-Trimethoxybenzanilide and *cis*-N-(3-indolyethyl)-N-3-methoxycyclohexylmethylacetamide, two representatives of other potential reserpine analogues, are approximately as active as tetrabenazine in depleting the 5-HT in the rat brain [635].

Contemporaneous administration of tetrabenazine and reserpine reduces the duration of reserpine sedation and produces a more prompt return of the brain 5-HT levels towards normal. The most convincing explanation of this observation, among the several offered by QUINN *et al.* [968], is that the short-acting tetrabenazine occupies at least some reserpine receptor sites in the brain, thus reducing the effects of the long-acting reserpine.

The depleting action of reserpine on brain 5-HT and norepinephrine is reinforced by previous administration of a new benzothiadiazine diuretic [1188], but is counteracted by pretreatment with chlorpromazine [949].

13.62 Action on 5-HT originating from exogenous 5-HTP

Administration of 5-HTP is capable of elevating the 5-HT level in brains depleted of the amine by pretreatment with reserpine. This elevation (from 0.05 to 0.35 $\mu\text{g/g}$, 1 hour following 50 mg/kg 5-HTP, i.v.) is, however, considerably less than that observed in control animals under the same conditions (from 0.5 to 1.7 $\mu\text{g/g}$ [1113, 172]).

13.63 Biochemical and pharmacological effects produced by the interaction of reserpine with MAO-inhibitors

The reserpine and tetrabenazine-induced decrease of the brain 5-HT level is partially antagonized in mice, rabbits, rats and guinea-pigs by pretreatment with MAO-inhibitors (e.g. iproniazid 100 mg/kg, β -phenylisopropylhydrazine 20 mg/kg) [168, 936, 938, 940, 66].

Similarly, the brain 5-HT content, depressed by previous administration of reserpine, shows enhanced recovery when MAO-inhibitors are administered subsequently. 3 mg/kg β -phenylisopropylhydrazine, for example, given intravenously to rabbits 18 hours after reserpine administration, elevates brain 5-HT from 0.05 to 0.23 and 0.37 μ g/g within 15 and 60 minutes, respectively [174, 1160].

In reality, according to SPECTOR *et al.* [1161b], MAO-inhibitors would not prevent reserpine from releasing 5-HT and noradrenaline from their binding sites, but would prevent the rapid decline in brain amines by inhibiting their metabolism after they are released. The fact that the disappearance of free amines from the brain is slow may be attributed to the difficulty with which these polar substances diffuse across the blood-brain barrier.

Surprisingly enough, raunescine (5 mg/kg, i.v.), a moderate 5-HT depletor, produces a further increase in the brain 5-HT level of rats pretreated with iproniazid: control rats 0.32 μ g/g; iproniazid pretreated rats 0.81 μ g/g; iproniazid pretreated rats given raunescine 1.10 μ g/g 5-HT [881].

Administration of MAO-inhibitors to animals pretreated with reserpine causes a transformation of the reserpine sedation into hyperactivity and excitement. This 'reversal' of reserpine action [168] has been described in greater detail in the paragraph which deals with MAO-inhibitors.

It is interesting to note that even when an increase of endogenous brain 5-HT is produced by drugs which are not listed among MAO-inhibitors, reserpine, while producing approximately the same percentage depletion of 5-HT, leaves more of the amine behind in the brain.

Thus in diphenylhydantoin pretreated rats the 5-HT remaining in the brain after reserpine is 0.24 instead of 0.1 μ g/g [144] and rats given LSD (0.13 mg/kg) after reserpine show a two-fold content of 5-HT in their brain, over reserpine controls [439].

13.64 Discussion

From their first experiments on brain 5-HT release by reserpine, BRODIE *et al.* concluded that 'reserpine acts centrally through release of serotonin' [933, 934] and that 'the primary action of reserpine is to impair serotonin binding sites so that they lose, in part, their capacity to retain 5-HT, which remains in a free, pharmacologically active form and presents to brain tissue a persistent low concentration of free serotonin'. It is this free serotonin that is considered to exert the actions attributable to reserpine [169, 170]. They further suggest that the 'reversal' of reserpine action, from sedation to hyperactivity produced by pretreatment with MAO-inhibitors depends on the fact that under these circumstances the serotonin, 'presumably in a free form, remains in a brain in high concentration' [168].

BRODIE's original view that the actions of reserpine on the CNS are mediated only or mainly through liberation of 5-HT and a change of the amine from a bound to a free form is tenaciously maintained by BRODIE's group, in

spite of formidable evidence to the contrary. Because of its extreme interest we shall discuss the problem in some detail.

a) Reserpine is by no means a specific 5-HT liberator. This is a fundamental point. In fact, reserpine also liberates adrenaline, noradrenaline [221, 592, 1114b] and dopamine [223b, 1086b], and it is highly probable that it liberates, to a greater or lesser extent, all other biogenic indolealkylamines (tryptamine, melatonin etc.) and phenylalkylamines (*o*-tyramine, *m*-tyramine, *p*-tyramine, phenylethylamine, octopamine, metanephrine, normetanephrine etc.) wherever they may be present in the mammalian tissue, including, of course, brain.

It has been claimed that reserpine is incapable of releasing histamine from nervous tissue. This may be true in some cases [1269] but the negative conclusions cannot be generalized. BERTACCINI [98], for example, has shown that reserpine produces a 90% reduction of the histamine contained in the optic ganglia of *Eledone moschata*. In contrast to the depleting effect on all the above amines, reserpine produces an increase in brain acetylcholine levels [914, 772].

This is not all. Reserpine interferes, in fact, with the storage not only of amines but of other active substances as well, because it produces a release of γ -aminobutyric acid [895], adenosine triphosphate (a compound probably possessing a specific role in the storage and release of biogenic amines) [653], ACTH [1307, 1045, 352, 657b] and luteotropin [62, 821]; and inhibits hypothalamic neurosecretion evoked by adrenaline [834], as well as thyrotropin secretion [843].

b) It is by no means the case that 5-HT depletion and pharmacological effects persist long after reserpine has disappeared from the brain, as claimed by HESS *et al.* [572], because studies with radioactive reserpine labelled with tritium and carbon-14 (0.1 mg/kg) have demonstrated that reserpine is present in the brains of guinea-pigs at an unchanging concentration throughout and beyond the entire period of observable pharmacological effects [1353, 1102, 1101].

c) While it is true that potentiation of barbiturate hypnosis by both reserpine and 5-HT is blocked by LSD [1107] it is also true that BOL, an equipotent inhibitor of 5-HT which lacks the central stimulatory effects of LSD, does not antagonize the barbiturate-potentiating action of reserpine although it blocks the 5-HT-induced potentiation of barbiturates to the same extent as LSD [1189].

Moreover, whereas LSD in very small doses enhances the potentiating effect of 5-HT on hexobarbital hypnosis in mice, it always blocks, at all dosage levels, reserpine-induced hypnosis [1035]. This rules out the existence of a common mechanism of action of reserpine and 5-HT as regards their barbiturate potentiation.

d) Experiments on ultracentrifuged brain homogenates have concordantly shown that following reserpine pretreatment there is a decrease in both free and bound 5-HT. In the case of catecholamines, the disappearance of amines following reserpine treatment is even more pronounced in the supernatant than in the particulate fraction [1282]. This does not fit the assumption that reserpine acts through a change of 5-HT in brain from a bound to a free form.

e) 5-HTP, the immediate precursor of 5-HT, is a very poor antagonist for reserpine sedation and ptosis, whereas not only L-DOPA but also *m*-tyrosine (the precursor aminoacid of the biogenic *m*-tyramine) have strong awakening effects upon mice treated with reserpine [222, 131].

Moreover, whereas DOPA given after iproniazid to reserpinized animals produces restoration of normal motor activity, 5-HTP given under the same conditions produces paralysis, tremors and clonic convulsions [388b].

Keeping in mind these clear, easily reproducible results, one hardly can accept the view of COSTA and PSCHIEDT [280] that the brain catecholamine content of rats is unrelated to the degree of ptosis and that, on the contrary, a positive correlation exists between eye closure and levels of brain 5-HT.

f) KÄRKI and PAASONEN [630] and PLETSCHER *et al.* [945] have brought evidence that the sedative effects of two amine-releasers, raunescine and benzoquinolizines, are associated with a selective loss of brain norepinephrine. They conclude that the tranquillizing action of reserpine and related compounds seems to be mediated rather by catecholamine than by 5-HT depletion.

Neither the experimental results nor the conclusions of the above investigators have been accepted by BRODIE *et al.* [874, 1183, 177] who tenaciously maintain that the tranquillizing action of reserpine is associated with decrease in brain 5-HT and not in brain noradrenaline. Evidence brought in support of this view is based mainly on two findings: α) low doses of Su 5171 (dimethylaminobenzoylreserpate, 0.25 mg/kg, i.v.) release in rabbits half the brain noradrenaline without releasing significant amounts of brain 5-HT and without eliciting sedation; large doses of Su 5171 release about 70% of both amines and induce marked sedation; β) rats or rabbits stressed by cold exposure are not sedated by reserpine: they show 55–75% depletion of noradrenaline, but only 20–30% of 5-HT; sedation appears only when the 5-HT level has declined by about 50% after the animals are brought back to room temperature.

In this evident conflict of opinion, a third, drastically negative position is taken by SHEPPARD and ZIMMERMAN [1102, 1353] who conclude that the pharmacological effects of reserpine are not correlated either with catecholamine or with 5-HT levels in the guinea-pig brain, but are due possibly to a direct effect on brain of the molecule of reserpine, *per se*. In fact, if it is true that the appearance of clear depressive effects following reserpine administration coincides with a 50% fall of brain 5-HT, it also is seen that almost all the sedative effects of reserpine disappear before the lowered 5-HT and noradrenaline levels begin to increase.

It is surprising that BRODIE himself [968] admits the possibility that the sedative effects of reserpine may not be due to a lack of brain amines. At the root of this admission is the observation that sedation produced in rabbits by simultaneous administration of tetrabenazine and reserpine disappears within 24 hours, when the brain 5-HT and norepinephrine levels are still 50 and 20% of normal, respectively.

g) It has been shown that in cats and dogs signs of excitation are lacking in spite of enormously elevated 5-HT levels produced by MAO-inhibitors, and

that in rabbits central excitation produced by the same drugs is temporally related to a rise in brain norepinephrine rather than to an elevation of brain 5-HT [1160]. Moreover, in the cat the only change made by iproniazid in the reserpine syndrome is the prevention of extreme miosis; no excitement, panting, tremor or pupillary dilation is produced. This is so in spite of the fact that the 5-HT content of cat brain after reserpine and iproniazid is high [1261]. Keeping in mind these essential data it is extremely improbable that the central excitatory effects elicited by reserpine in iproniazid pretreated animals may be ascribed, as postulated by BRODIE *et al.* to the accumulation in brain tissue of free 5-HT.

h) KUNTZMAN *et al.* [684c] affirm that the combined use of α -methyl-*m*-tyrosine (500 mg/kg, i.p.) and reserpine gives results compatible with the view that noradrenaline increase in brain is associated with excitation and 5-HT increase with sedation. More precisely, they lay emphasis on the fact that α -methyl-*m*-tyrosine, which provokes in mice a transient and partial lowering of brain 5-HT and a complete and persistent depletion of brain noradrenaline, does not produce any sedation.

It is clear that this piece of evidence has lost most of its value since HESS *et al.* [574c] demonstrated that the persistent depletion of noradrenaline seen after administration of α -methyl-*m*-tyrosine is not due to inhibition of noradrenaline biosynthesis, but to some other mechanism, possibly a blockade of the noradrenaline binding sites. In sharp contrast to noradrenaline, dopamine returns to normal levels as quickly as 5-HT following administration of α -methylaminoacids.

i) The picture of the central actions of reserpine (alone or with MAO-inhibitors) on the one hand, and of 5-HT or 5-HTP (alone or with MAO-inhibitors) on the other, offers such an inextricable pattern of convergencies and divergencies that it is extremely difficult to draw from it any conclusion about the possible interference of 5-HT with the mechanism of action of reserpine. On the whole, data are accumulating which show that there are striking differences in the central action of reserpine on the one hand and of 5-HT and 5-HTP on the other [1119, 179, 116, 109, 1070b].

k) Last but not least it should be remembered that reserpine depression may be removed instantly by amphetamine, D-deoxyephedrine, LSD, methylphenidate and imipramine which, so far as we know at the present time, do not change the brain 5-HT level either in normal or in reserpinized animals.

These data do not appear to be consistent with the hypothesis that sedation produced by reserpine is mediated through 5-HT [194].

To sum up, whereas there is no doubt that reserpine is a powerful 5-HT depletor, it is necessary to admit that not simply a lack of 5-HT but a very complex alteration in the availability and equilibrium of various biogenic amines and other biochemically essential compounds is at the root of the central and peripheral actions of this tranquillizing drug.

At present, major interest is focussed on the catecholamines as being mainly responsible for the action of reserpine. But, independently of the predominant interference which may be attributed to one amine rather than to another in

the pharmacological effects of reserpine, nearly all investigators reject the theory that the alkaloid acts through a shift of the amines from a bound to a free form, i.e. through an excess of free amines. The opinion shared by the great majority of research workers is that a lack, rather than an excess, of free, physiologically active amines is responsible for the effects of reserpine.

This criticism is not intended to minimize the enormous importance of the biochemical data gathered through the use of reserpine, but only to point out the complexity of the problem concerning the mechanism of action of this drug and the advisability of avoiding, if possible, theories and hypotheses which all too often must be regarded as mere speculation.

13.7 Release of 5-HT from the CNS by nervous stimulation

It is evident that demonstration of a release of 5-HT during spontaneous or evoked activity of the CNS would represent a good piece of evidence in favour of the hypothesis that 5-HT is a chemical mediator.

ANGELUCCI [18] detected very small amounts of a substance resembling 5-HT in the perfusate of the spinal cord of a frog subjected to cutaneous electric stimulation. He suggests that the liberation of 5-HT may play a part in spinal reflexes.

BENETATO *et al.* [88] found that excitation of the central ends of the cut vagus and Hering nerves produces the appearance of 5-HT, acetylcholine and adrenaline in the perfusate of an isolated dog head. Addition of atropine to the perfusion liquid has no effect on the 5-HT output, but the addition of reserpine increases it conspicuously. However, excitation of the vagus nerves in reserpine-pretreated dogs no longer produces liberation of 5-HT. Following 5-HT depletion of brain tissue, the vasoconstrictor responses elicited by stimulation of the central ends of the vagus disappear, but they can be restored by intrarachideal injection of 5-HT. On the contrary, the presence of 5-HT is not necessary for the appearance of parasympathetic reflexes.

It seems that until they are confirmed, the above results should be accepted with reservation.

13.8 Miscellaneous factors affecting the 5-HT content of the CNS

Drugs. In rats, a whole series of anticonvulsant compounds—diphenylhydantoin 100 mg/kg i.p., Mesantoin® 200 mg/kg; trimethadione 150 mg/kg; paramethadione 200 mg/kg; phensuximide 450 mg/kg; phenylacetylurea 500 mg/kg; primidone 500 mg/kg; phenobarbital 125 mg/kg; prominal 125 mg/kg; sodium bromide 1000 mg/kg—have been shown by BONNYCASTLE *et al.* [144, 145, 19b] to cause a significant elevation of brain 5-HT levels (from 0.34 to 0.48–0.70 $\mu\text{g/g}$). This increase only occurs in brain tissue and has not been observed in spleen, upper small intestine or blood.

The above results could not be confirmed by GESSA and COSTA [474b], who were unable to find any increase of brain 5-HT and noradrenaline following phenobarbital and pentobarbital administration. GESSA and COSTA suggest that

the reasons for the discrepancy between their own results and those of BONNY-CASTLE and co-workers lie in the inaccuracy of the bioassay procedure employed by the latter investigators. However, this point of view seems somewhat arbitrary, as GESSA and COSTA have not carried out, as some people would have expected, parallel assays with spectrofluorometric and biological methods. Up to the present, the alleged superiority of spectrofluorometric analysis over bioassay in the quantitative estimation of 5-HT has by no means been demonstrated.

A small increase in rat brain 5-HT has also been observed after ethanol, morphine and bulbo-capnine. No consistent change, on the contrary, has been obtained with amphetamine, azacyclonol, meprobamate, apomorphine, CO₂, Dibenamine®, ethyl ether, hexobarbital, LSD, mescaline, pipradol, yohimbine, 48/80, heparin and hydrocortisone [880] nor with parathion and similar organophosphorus insecticides [1086c].

Triethyltin (5–10 mg/kg, i.p.) reduces the concentration of 5-HT in the rat brain by approximately 30–40%, and causes a similar depletion of noradrenaline. However, it seems difficult to establish a causal relationship between tranquillization produced by the drug and the reduction of brain amines [843b].

Thyroidectomy apparently has no effect on brain 5-HT. However, both L-thyroxine (daily injections of 0.25–0.5 mg) and propylthiouracil (0.075% solution in drinking water) produce a significant increase in the 5-HT concentration of the rat brain [1204].

Like thyroidectomy, adrenalectomy and hypophysectomy produce none or only small changes in brain 5-HT; ACTH administration after hypophysectomy decreases brain 5-HT [1336, 1205].

In rabbits the following drugs have been found to be inactive on the brain 5-HT level: phenobarbital (100 mg/kg i.v.), barbital (200 mg/kg in divided doses), morphine (20 mg/kg), scopolamine (50 mg/kg), D-amphetamine (70 mg/kg), diphenhydramine (35 mg/kg), LSD (0.12 mg/kg), mescaline (75 mg/kg), chlorpromazine (10 mg/kg), azacyclonol (75 mg/kg), yohimbine (2 mg/kg), cortisone (26 mg/kg) and several non-tranquillizing *Rauwolfia* alkaloids [171].

Ethanol, on the contrary, produces, when given intravenously in saline solution in doses of 2 g/kg, a marked depression of brain 5-HT and noradrenaline levels. The maximum decrease (50%) is observed 30 to 120 minutes after the ethanol infusion, but approximately 100 hours are required before the initial levels are regained [524]. Lower doses of alcohol given for 7 days have the same depleting effect [525].

Insulin, ether and ephedrine cause no change in the 5-HT content of the dog brain, but amphetamine (20–30 mg/kg) lowers it to approximately one half [876].

It may be seen that results obtained by the various investigators are not completely concordant and, what is more important, that discrepancies concern drugs particularly interesting from a theoretical point of view, such as antiepileptic drugs (phenobarbital, diphenylhydantoin), ethanol and amphetamine. Although these discrepancies may depend upon different animal species

and different experimental conditions, they suggest that caution should be used in drawing speculative conclusions of general significance from experimental evidence not sufficiently consistent and extensive.

For example, the existence of a possible relationship between an increase in brain 5-HT and the specific action of anticonvulsant drugs has been questioned by PROCKOP *et al.* [960], who have found that doses of 25–75 mg of diphenylhydantoin which block in rats the tonic extension component of the electroshock do not increase the brain amines.

Total irradiation of the body provokes in rats a significant decrease of 5-HT in the hypothalamus (from 1.48 to 0.30 $\mu\text{g/g}$) [974].

Electroshock and convulsant metrazol or fluoroacetate doses produce in rats either a small increase (30%) in cerebral 5-HT or no effect whatever, leaving unaffected the amine content of blood, gastrointestinal tract and other tissues [96, 145, 454e].

Similarly, convulsions evoked by metrazol do not exert any influence upon the rate of 5-HT release from the brain produced by reserpine [454e].

Pyridoxin-deficiency produces in chickens, probably by its negative interference in 5-HTP decarboxylation, a 70% reduction in brain 5-HT level [1336]. However, in B_6 deficient rats there is only a small decrease in the brain 5-HT concentration, and the correlation of this decrease with behaviour of B_6 deficient animals is not clear [1228].

Neither *starvation* nor *thiamine deficiency* has any effect on the 5-HT content of the rat brain [454d].

Stress due to the presence of a dog has caused in a hare at first a transient increase (from 0.075 to 0.1 $\mu\text{g/g}$), and then a decrease (down to 0.04 $\mu\text{g/g}$) in brain 5-HT. Concluding from these meagre results that 5-HT is highly engaged in the adaptation syndrome seems to be frankly excessive [830].

Removal of the large intestine produces in rats a significant decrease in brain 5-HT concentration. The reason for this phenomenon is obscure [99].

13.9 Indolealkylamines and the CNS in human beings

13.91 Central effects of 5-HT

On the whole, the statement of MARAZZI [787] is still valid that 'there have been, thus far, no documented reports of serotonin-induced mental disturbances in man that are clearly separable from the natural anxiety initiated by the profound peripheral effects such as circulatory disturbance, other autonomic effects and emesis'.

Of course, true central effects might be expected from the administration of 5-HTP, provided that sufficient amounts of the aminoacid are given. However, here again things are complicated by the possibility that the central actions of the 5-HT originating from the precursor aminoacid within the brain tissue are masked or exaggerated by the peripheral actions of the 5-HT originating from extracerebral decarboxylation of 5-HTP.

Low doses of either 5-HT (2-6 mg, i.v.) or 5-HTP (10 mg, i.v.) did not produce significant changes in the EEG in man [1046, 107, 916d, 691].

Similarly, intravenous administration of 80-150 mg 5-HTP to human subjects failed to cause definite psychological effects or any consistent change in the reaction to LSD [662b]. At the same dosage, 5-HTP was generally ineffective in schizophrenic patients, too [662c].

13.92 Central effects of indolealkylamines other than 5-HT

Bufotenine. FABING *et al.* [389, 389b] first observed that bufotenine given intravenously in man (0.06-0.25 mg/kg, over 3 to 20 minutes) produces, besides intense vegetative effects, visual disturbances, colour hallucination, nausea and mental confusion, together with mild disorientation. These phenomena are followed by gradual relaxation, lassitude and withdrawal. Return to usual mental awareness is slow and there is a significant euphoria lasting for several hours.

TURNER and MERLIS [1216] investigated the action of bufotenine mainly in schizophrenic patients. Despite profound EEG changes, loss of consciousness and intense peripheral actions imitating those of 5-HT—flushing of the face, lacrimation, tachycardia, tachypnoea, rise of blood pressure, mydriasis, need to defecate or abdominal distress, constriction of the chest—no hallucinations are provoked and the patients' psychosis is neither aggravated nor relieved by the injection of bufotenine (up to 20 mg, i.v.).

N,N-dimethyltryptamine and *N,N-diethyltryptamine*. The model psychosis produced in man by the administration of either N,N-dimethyltryptamine [1026] or N,N-diethyltryptamine [152, 153] (0.65-1.50 mg/kg i.m.) resembles moderate mescaline intoxication and, to a lesser degree, the effects of LSD. The psychosis lasts less than one hour.

In schizophrenic patients the vegetative symptoms appear later than in normal volunteers and are less intense; hallucinations are also lacking in schizophrenics, in contrast to normal subjects [1216, 152, 153].

SZARA *et al.* [1186, 1186b] suggest that the psychiatric effects of the above tryptamines are due to their metabolically-formed 6-hydroxy derivatives rather than to the tryptamines themselves.

DL- α -Methyltryptamine. In a dose of 20 mg, given by mouth, this drug produces a subjective action similar to 50 μ g of LSD. The effects appear later, however, with a lag of up to 3 hours [854b].

Psilocybin. (O-phosphate of N,N-dimethyl-4-HT). Psilocybin has been thoroughly studied in its somatic and psychic effects by DELAY *et al.* [311, 312, 313, 314] and by other investigators. The drug was administered *per os* (0.06-0.12 mg/kg), parenterally (5-14 mg) and sublingually (5-10 mg).

Somatic symptoms produced by psilocybin include lightheadedness, nausea, photosensitivity, weakness, increased deep tendon reflexes, mydriasis and blood pressure changes. The main psychic symptoms are changes in mood characterized by euphoria or dysphoria, difficulty in concentration and thinking, marked sensory perceptual distortion, alterations in body image and, sometimes, hallucinations [612, 983, 591b].

In comparison with psilocybin, mescaline has been found to be more hallucinogenic and LSD more depersonalizing [312, 313, 314]. The effects of LSD are longer-lasting and 100–150 times as great as those of psilocybin [612].

The therapeutic usefulness of psilocybin [311] seems to be limited to facilitation of psychotherapy.

BAS (Benzyl analogue of serotonin). According to WILKINS and HOLLANDER [1317] and RUDY *et al.* [1012] BAS is a tranquillizing agent producing at daily doses of 50–200 mg tranquillization which in many ways resembles that caused by reserpine. However, the beneficial effects are marred by remarkable untoward effects.

Intravenous injection of 2 mg/kg BAS in chronic psychotic patients produces no significant changes in the EEG [981].

DL-4-Hydroxytryptophan. In oral doses of 3–15 mg/kg the aminoacid failed to produce, in a normal subject, any obvious central effect [381b].

L-Tryptophan. L-tryptophan, a common dietary aminoacid, does not display, *per se*, any central effect. However, when administered in oral doses of 20–50 mg/kg to patients pretreated with β -phenylisopropylhydrazine (25 mg/day for 4 days) the aminoacid apparently produces changes in mental status resembling ethanolic intoxication, ataxia, euphoria, drowsiness, hyperreflexia and ankle clonus.

This observation by OATES and SJOERDSMA [868d] has been confirmed by POLLIN *et al.* [952b] in schizophrenic patients maintained on iproniazid (50–150 mg) for several days. However, surprisingly enough, marked alterations in behaviour in some patients were associated not only with the administration of tryptophan (15 g daily), but also with that of methionine (20 g daily).

It is hardly necessary to say that this result impairs the validity of the hypothesis that central actions of MAO-inhibitors are mediated only through amines originating from tryptophan.

13.93 5-HT in psychiatric and neurological disorders

5-HT has been involved in the pathogenesis of some of the symptoms of phenylketonuria, of schizophrenia and of other mental and neurological disorders.

Phenylketonuria. PARE *et al.* [896] first demonstrated that the urine of phenylketonuric children contains less than 50% of the 5-HIAA occurring in normal urine. Concomitantly, serum levels of 5-HT are 0.06 μ g/ml, instead of the normal 0.124 μ g/ml.

The investigators conclude that the above findings are compatible with the view that there is in these patients a disturbance in tryptophan metabolism, which results in a diminished production of 5-HT, and that impaired 5-HT synthesis might contribute to the cause of mental deficiency in patients with phenylketonuria.

DAVISON and SANDLER [306] advanced the hypothesis that the decreased 5-hydroxyindole production is caused by a relative inhibition of 5-HTP decarboxylase by excess phenylalanine metabolites. In fact, following administra-

tion of 5-HTP the urinary output of 5-HIAA and 5-HT is lower in children with phenylketonuria than in control children [897], and, on the other hand, both serum 5-HT levels and urinary excretion of 5-HIAA increase after feeding low phenylalanine diets [897, 51, 89].

A disturbance in tryptophan hydroxylation does not seem to play an important role, since oral administration of L-tryptophan (100 mg/kg) to untreated phenylketonuric patients is followed by an immediate increase in 5-HIAA excretion [897].

However, parenteral administration, in a 3-month-trial, of 5-HTP to children suffering from phenylketonuria failed to produce any improvement, thus giving no support to the hypothesis that a decrease in 5-hydroxyindole production is the cause of the mental defect seen in the disease [898].

Pooled cerebrospinal fluid of non-premedicated phenylketonuric patients contains less than $0.005 \mu\text{g}$ 5-HT per ml, that of patients pretreated with MAO inhibitors less than $0.05 \mu\text{g/ml}$ [916b].

In experimentally produced phenylketonuric rats the rise in urinary phenylpyruvate excretion caused by supplementation of the diet with both phenylalanine and tyrosine coincides with a decrease of 5-HIAA in the urine, and the increase of phenylalanine in plasma with a decrease of plasma 5-HT (from $0.81\text{--}0.88$ to $0.42\text{--}0.58 \mu\text{g/ml}$). This further supports the hypothesis that the disturbance of 5-hydroxyindole metabolism in phenylketonuria is secondary to the increase in phenylalanine [602b].

Schizophrenia. It has been claimed by some investigators that schizophrenic patients excrete less 5-HIAA than normal subjects [736, 58]; that oral administration of L-tryptophan influences the urinary output of 5-HIAA in schizophrenics in a way different from fit subjects [1351, 58, 704]; that recovery of exogenous 5-HT as 5-HIAA is higher in schizophrenics than in normal subjects [199]; and finally, that there is in the urine of schizophrenic patients an abnormal presence of unidentified indole compounds [736, 347, 199, 813b].

All these claims have been contradicted regularly by a more numerous group of investigators.

In fact, no significant differences in the excretion of 5-HIAA between chronic or acute schizophrenic patients and non-psychotic fit subjects could be observed by FELDSTEIN *et al.* [399], nor by ROBINS *et al.* [987], SANO *et al.* [1042, 1043], CURZON [290b], BUSCAINO and STEFANACHI [199]; no differences in the urinary output of 5-HIAA following a tryptophan load could be demonstrated by KOPIN [674] between normal and schizophrenic subjects; and finally, no correlation could be established between schizophrenia and positive indole colour reactions in the urine by HELLINGER and PACTER [562].

It should be added that both the urinary excretion of 5-HT and the level of 5-HT in blood [401, 623b] and in liquor [1217] of schizophrenic subjects do not differ significantly from those of normal subjects, and that electroshock therapy does not alter either the 5-HT concentration in blood or the urinary excretion of 5-HIAA [516, 662].

The significance of the above data, which do not indicate any causal relationship between 5-HT and schizophrenia, has been strengthened by the result of therapeutic trials directed on the one hand to the removal of an eventual deficiency of brain 5-HT and on the other to antagonizing eventual excess 5-HT.

Administration of 5-HTP alone, or in combination with BAS and with iproniazid (in order to increase brain 5-HT) was completely unsuccessful [899, 162, 400, 582, 662b], as it was the administration of BOL (in order to antagonize 5-HT), and patients did not show any sort of clinical change [1214].

Similarly, at variance with the positive results of FOULKS *et al.* [436], 5-HT also has proved to be of no value in the treatment of schizophrenia in the hands of several groups of investigators [237, 267, 691, 916d].

In conclusion, there is as yet no convincing evidence that excess or deficiency of brain 5-HT, caused by a disturbed metabolism of the amine, may participate in the pathogenesis of schizophrenia, nor that 5-HTP or 5-HT may be of therapeutic benefit in the disease [134, 396, 650, 648, 400].

Other diseases of the CNS. The more complete study of 5-hydroxyindoles in mental deficiency has been carried out by PARE *et al.* [899b]. They found that the serum 5-HT concentration is above normal ($0.15 \mu\text{g/ml}$) in cerebral palsy ($0.35 \mu\text{g/ml}$), maternal rubella ($0.39 \mu\text{g/ml}$), gargoylism ($0.27 \mu\text{g/ml}$) and in 'cerebral lipidosis' ($0.49 \mu\text{g/ml}$), and normal in mongolism, hypertelorism and epilepsy. The urinary excretion of 5-HIAA, in its turn, is raised in all the above syndromes, except epilepsy.

In a patient with an unusual neurologic disease characterized by ataxia, vasomotor instability and dysautonomic seizures, SOUTHERN *et al.* [1148] found abnormally high serum 5-HT levels ($4\text{--}30 \mu\text{g/ml}$) without a corresponding increased urinary excretion of 5-HIAA.

It is suggested that certain cases of dysautonomic seizures or ataxia may be correlated with disorders in indole metabolism, possibly dependent upon decreased utilization of endogenous stores of 5-HT.

MAGISTRETTI and PEIRONE [766b] hypothesize that the neurological and psychiatric manifestations of acute and subacute carbon disulphide poisoning in man may be due, at least partly, to the accumulation of 5-HT in brain. This assumption is based on the meagre evidence that carbon disulphide is capable of blocking MAO, both *in vitro* and in the rat organism. No estimation of brain 5-HT in intoxicated animals has been carried out.

Out of nine examined cerebral tumours, only two contained detectable amounts of 5-HT: they were a retinoblastoma ($3 \mu\text{g/g}$) and a ganglioneuroma ($1.3 \mu\text{g/g}$) [512b].

Moderate or marked elevations of cerebrospinal fluid 5-HT have been found in a number of subjects with a variety of primary brain tumours. In a case of carcinoid metastasis to the choroid of the eye, SOUTHERN [1149] found $0.035 \mu\text{g/ml}$ 5-HT in cerebrospinal fluid.

5-HT in the liquor is also elevated in tuberculous children ($0.2\text{--}3 \mu\text{g}$ instead of $0.03\text{--}0.1 \mu\text{g/ml}$) and it has been claimed that the clinical course of the disease closely follows the 5-HT content in cerebrospinal fluid [13].

13.10 Concluding remarks

After having reviewed and discussed in the preceding pages the main available data on 5-HT and related compounds in the CNS, it now seems that the time has come to put the fundamental question concerning 5-HT in the brain: what is the biological significance and what is the function of this amine within the CNS?

Leaving aside the acceptable, prudent claim of GADDUM [26, 451] and of WOOLLEY [1324, 1334, 1318c] that 5-HT is needed for normal functioning of the brain, several investigators have not hesitated to advance much more binding and precise theories concerning the function of brain 5-HT.

Among them, the two which have attracted the most attention and have stimulated the most work, are that of BRODIE's group and that of MARAZZI and HART.

According to BRODIE, 5-HT [169, 170, 1112] is a chemical transmitting agent in the parasympathetic division of the central autonomic nervous system, i.e., a neurohormone involved in the HESS 'trophotrophic' system, which normally interferes with the regulation of centres in the brain that involve wakefulness, temperature regulation, blood pressure regulation, and a number of other autonomic functions. 5-HT may act preferably on the brain centres in the hypothalamus and other subcortical centres. Whereas normal amounts of 5-HT may facilitate conduction and transmission of nervous impulses, excess 5-HT is considered to block conduction by inducing a persistent state of depolarization.

This is not all, as BRODIE gives a tentative explanation of the mechanism of action of many other substances acting on the CNS.

Noradrenaline, in fact, could be the chemical mediator of the opposing 'ergotropic' system. Reserpine, as already stated, could act by releasing 5-HT or, better, by changing brain 5-HT from a bound to a free form. Chlorpromazine could produce its sedative effects by blocking the action of norepinephrine. Finally, LSD could cause hallucinations through a block of nerve conduction brought about by interference with the normal depolarizing action of 5-HT released at synaptic junctions.

MARAZZI and HART [786, 787] agree with BRODIE that 5-HT is a chemical transmitter within the CNS but, according to them it is a negative transmitter, to be listed among the adrenergic cerebral neurohumors. Because 5-HT is about 20 to 25 times as potent as adrenaline in causing cerebral synaptic inhibition, it would be a better candidate than the catecholamines for the role of inhibitory neurohumor.

MARAZZI and HART further affirm that a disturbance of adrenergic or related cerebral neurohumoral mechanisms appears to be implicated in the action of hallucinogens, such as mescaline and LSD, and that the resulting lack of balance in the reciprocal relationship between adrenergic inhibition and cholinergic excitation in the most susceptible cerebral synapses might be an underlying mechanism in mental disturbances.

The single points of BRODIE's theory, which is mainly based on studies with MAO-inhibitors and 5-HT-liberators, have been discussed in detail elsewhere. Here it is sufficient to note that the concept that 5-HT exerts in the brain effects which are generally opposed to those possessed by the catecholamines is, at present, the most persuasive, as it is also in keeping with some reliable experimental results demonstrating that very often DOPA and 5-HTP produce opposite central effects [493, 855].

Concerning MARAZZI's theory, the candidature of 5-HT for the role of inhibitory neurohumor has suffered a hard blow from MARAZZI himself. In fact, GILFOIL and MARAZZI [491] have demonstrated that histamine, too, is a potential cerebral synaptic inhibitor on transcallosally evoked potentials in the optic cortex, of the order of potency of 5-HT *or better*. Of course, one may well expect that the list of 'new cerebral synaptic neurohumoral inhibitors', is destined to lengthen, demonstrating the uselessness of premature speculations.

After the first enthusiasm for and even infatuation with 5-HT, 5-HT releasers and MAO-inhibitors, a more critical position is now being taken up by an increasing number of authoritative research workers.

There are even investigators who raise some doubt about the actual importance of 5-HT and catecholamines as chemical mediators in the CNS. The most important investigation in this respect should be briefly reported.

To see whether the brain which has been depleted of 5-HT and catecholamines still discharges normally into the preganglionic sympathetic fibres, IGGO and VOGT [609, 610] compared preganglionic activity in normal and in reserpine-treated cats.

Preganglionic impulses were recorded in fine strands of the cervical sympathetic nerve and discharges were brought about by acoustic stimuli. The result arrived at is that preganglionic sympathetic discharge, both spontaneous and in response to stimuli, is not fundamentally altered by doses of reserpine which abolish all peripheral sympathetic activity and cause severe depletion of the stores of noradrenaline and of 5-HT in the brain.

Thus, the conclusion is permissible that the stores of these amines are not essential for normal sympathetic discharge and for normal response of the autonomic centres to external and internal stimuli.

The writer's opinion is that biogenic amines must certainly play an important and perhaps essential role in the normal functioning of the brain and that quantitative or, even more, qualitative changes in the biosynthesis and metabolism of these amines may contribute to the derangement of the normal function of the CNS. However, for the present, this is all that can be said about these compounds.

The routine use of MAO-inhibitors has permitted, and will permit, the demonstration in biological materials of a number of otherwise undetectable amines, the presence of which in the mammalian organism had been barely suspected. It is an easy prophecy to predict that the majority of amines which are found or will be found in urine or extracerebral tissues will be detected also in neuronal tissue.

Until we have a more complete list of the biogenic amines present in the CNS and have more information concerning the biochemical and functional correlations existing among these compounds, it seems futile to attempt to ascribe to a *single* amine the responsibility for the pharmacological or therapeutical effects of drugs which will probably act, directly or indirectly, on the biosynthesis, storage or destruction of *all* amines contained in the brain.

In conclusion, it appears that more numerous, precise and concordant analytical data must be gathered before attempting a synthesis.

The small and agile molecules representing the biogenic amines possess all the qualities required for acting as chemical transmitters or neurohumors, and it is certain that problems concerning indolealkylamines, phenylalkylamines and imidazolealkylamines will represent for years to come one of the most fascinating topics for study in the field of neurochemistry and psychopharmacology.

Acknowledgement

I am indebted to and wish to thank Mr. P. COOK (Cambridge School of Parma) for his kind assistance in the linguistic correction of the manuscript.

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The survey of the literature pertaining to this review was concluded in March-April 1961.